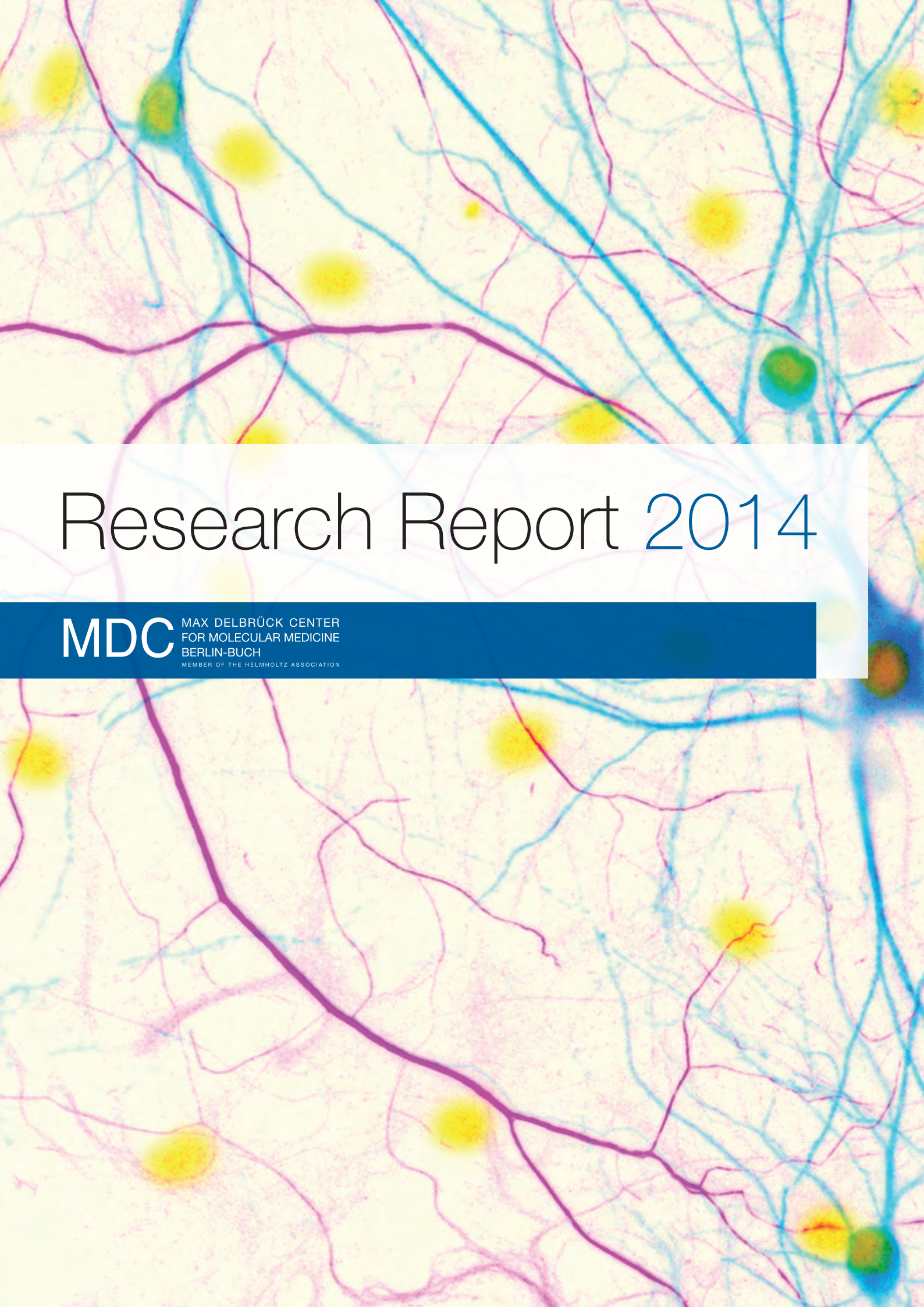


Research Report 2014

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE BERLIN-BUCH
MEMBER OF THE HELMHOLTZ ASSOCIATION

Research Report 2014

(covers the period 2012-2013)

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Neuronal cells (blue-green) where stimulated through gene transfer to produce therapeutical proteins protecting the brain from epilepsy. Axons are depicted in pink, nuclei in yellow.
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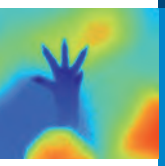
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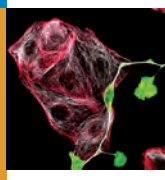
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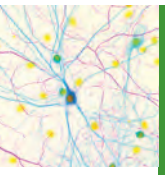
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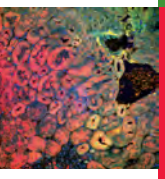
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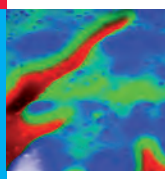
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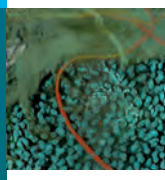
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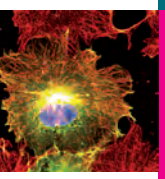
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Introduction by the Scientific Director

Vorwort des Wissenschaftlichen Vorstands

This research report looks back to two eventful years at the Max Delbrück Center. We have not only launched the Berlin Institute of Health, but also have co-founded the German Centre for Cardiovascular Research (DZHK). In 2012, we celebrated our 20-year anniversary and we have set the course for the MDC to open for the first time a second major site in the heart of Berlin.

With all this, the MDC looks ahead to major changes in many respects. In the coming two years, laws will come into effect that will regulate the establishment of the Berlin Institute of Health as a new public body and which thus will regulate also the future of our center. The MDC and the Charité – Universitätsmedizin Berlin will both become member institutions of the Berlin Institute of Health (BIH). This way, we will institutionalize our partnership with the Charité that we have been maintaining since our founding in 1992. At the same time, both parts of the new institute will maintain their respective identities and their very own range of tasks. In the case of the MDC, these tasks are biomedical basic research and disease-oriented research that is embedded into the strategic research of the Helmholtz Association, Germany's largest research organization.

The driving force in conceiving the BIH and in developing the funding programs was science with its desire to close the gap between basic research and clinical application research. The approach that was used here focuses on systems medicine: away from the traditional notion of "one disease – one mechanism". Rather, we know today that a disease can be caused by various mechanisms and their interactions, just as a given molecular mechanism can be involved in causing several different diseases ("one disease – many mechanisms" and "one mechanism – many diseases"). The Charité and the MDC perceive themselves to be predestined for this project as they have been closely cooperating with each other from the very start. In 1992, translation, that is, the fastest possible transfer of basic research insights into clinical application, formed part of

Dieser Forschungsbericht blickt auf zwei ereignisreiche Jahre am Max-Delbrück-Centrum zurück. So haben wir nicht nur das Berliner Institut für Gesundheitsforschung/ Berlin Institute of Health auf den Weg gebracht, sondern auch das Deutsche Zentrum für Herz-Kreislauf-Forschung mitgegründet. Wir haben 2012 unser 20-jähriges Bestehen gefeiert und wir haben die Weichen dafür gestellt, dass das MDC erstmals einen zweiten großen Standort im Herzen Berlins eröffnen wird.

Das MDC steht damit in vielerlei Hinsicht vor großen Veränderungen. In den kommenden beiden Jahren werden Gesetze auf den Weg gebracht, die die Gründung des Berliner Instituts für Gesundheitsforschung als neue Körperschaft des öffentlichen Rechts und damit auch die Zukunft unseres Zentrums regeln. Das MDC und die Charité – Universitätsmedizin Berlin werden jeweils Gliedkörperschaften des Berlin Institute of Health (BIH) werden. Auf diese Weise institutionalisieren wir die seit unserer Gründung 1992 bestehende Partnerschaft mit der Charité. Beide Teile des neuen Instituts werden dabei ihre jeweils eigene Identität und ihre ureigenen Aufgaben behalten. Im Falle des MDC ist das die biomedizinische Grundlagenforschung und die krankheitsorientierte Forschung, die eingebettet ist in die strategische Forschung der Helmholtz-Gemeinschaft, Deutschlands größter Forschungsorganisation.

Treibende Kraft bei der Konzeption des BIH und der Ausarbeitung der Förderprogramme war die Wissenschaft mit ihrem Wunsch, die Lücke zwischen Grundlagenforschung und klinischer Forschung zu schließen. Der Ansatz ist dabei systemmedizinisch: Er geht weg von der traditionellen Vorstellung „eine Erkrankung – ein Mechanismus". Vielmehr wissen wir heute, dass eine Erkrankung durch verschiedene Mechanismen und deren Wechselwirkungen verursacht werden kann, ebenso wie andererseits ein molekularer Mechanismus an unterschiedlichen Erkrankungen beteiligt ist („eine Erkrankung – viele Mechanismen" und „ein Mechanismus – viele Erkrankungen"). Charité und MDC sehen sich als prädestiniert für dieses Vorhaben an, kooperieren sie doch seit jeher



Photo: Steffen Weigelt/MDC

Video: Profile of the MDC

Prof. Dr. Walter Rosenthal

the founding charter of the MDC. Already in 2007, against this background, we established together with the Charité the Experimental and Clinical Research Center (ECRC), which has been headed by Fred Luft since that time. The BIH will build on the experiences we have gathered with the ECRC and it will add further value to the site of Berlin-Buch.

I would like to use this opportunity to express my gratitude to policy-makers on both a federal government and federal state level who have placed such great trust in us and whose initiative for establishing the new institute matched the scientific plans of the Charité and the MDC. I would also like to thank the Helmholtz Association, which finances the BIH during the first two years. And my special thanks go to entrepreneur Johanna Quandt, who will provide the new institute with a total of 40 million Euro in the context of her private excellence initiative. Thanks to this initiative, which is administrated by the Charité Foundation, we have managed to win over Nobel Prize laureate Thomas Südhof to come to Berlin for a BIH project as a first visiting scientist (BIH Visiting Fellow).

The German Centre for Cardiovascular Research (DZHK) started operations in April 2012. It is part of an initiative by the German Federal Ministry of Education and Research, which has initiated health research centers and provides funding for these. The MDC is involved in the DZHK by way of a seat on the

eng miteinander. Die Translation, also die möglichst rasche Übertragung der Erkenntnisse aus der Grundlagenforschung in die Klinik, gehörte 1992 zum Gründungsauftrag des MDC. Vor diesem Hintergrund haben wir mit der Charité bereits 2007 das Experimental and Clinical Research Center (ECRC) gegründet, dem Fred Luft seither vorsteht. Das BIH wird auf den Erfahrungen aufbauen, die wir mit dem ECRC gesammelt haben, und es wird den Standort Berlin-Buch weiter aufwerten.

Ich möchte an dieser Stelle Dank sagen an die Politik in Bund und Land, die uns mit einem großen Vertrauensvorschuss ausgestattet hat und deren Initiative zur Gründung des neuen Instituts zu den wissenschaftlichen Plänen von Charité und MDC passte. Ebenfalls bedanken will ich mich bei der Helmholtz-Gemeinschaft, die das BIH in den ersten beiden Jahren finanziert. Und mein ganz besonderer Dank gilt der Unternehmerin Johanna Quandt, die mit ihrer privaten Exzellenzinitiative insgesamt 40 Millionen Euro für das neue Institut zur Verfügung stellen wird. Dank dieser Initiative, die von der Stiftung Charité administriert wird, ist es uns gelungen, den Nobelpreisträger Thomas Südhof als ersten Gastwissenschaftler (BIH Visiting Fellow) für ein BIH-Projekt nach Berlin zu holen.

Das Deutsche Zentrum für Herz-Kreislauf-Forschung DZHK nahm im April 2012 seine Arbeit auf. Es ist eingebettet in eine Initiative des Bundesministeriums für Bildung und Forschung,

board as well as by way of scientific projects and the administration of funding.

In the past two years, we have also paved the way for the MDC's Berlin Institute for Medical Systems Biology (BIMSB) to be allocated a site in Berlin-Mitte. It is part of the future concept of the Humboldt Universität (HU) and thus has contributed to the HU winning its status of "University of Excellence". Together with us and the Charité, the HU has established the Integrative Research Institute (IRI) for the Life Sciences. The aim of the IRI for the Life Sciences is to further develop the HU's Campus Nord to become an influential science park in the heart of Berlin; the BIMSB is expected to move into a new building on the site as of 2018.

Moreover, in 2013 we established the European EU-Life alliance with twelve partnering institutions in the life sciences. It is intended to facilitate the exchange of information and career moves within Europe and to provide the life sciences with more political impact in dealing with European Union committees and bodies.

The basis for all these successes and developments in the world of science policy is the excellent work carried out by our scientists. In 2012, more than 30 international reviewers assessed the Max Delbrück Center. Our center received the best possible mark of "outstanding". This success of our working groups is reflected also in the number of publications and in individual awards. For instance, Nikolaus Rajewsky was awarded the Leibniz Prize 2012 and, since the publication of the last research report, we have attracted two new ERC grants. This makes the total number of such projects at the MDC that each are funded with several million Euro by the European Research Council ERC ten (originally eleven; the ERC grant winner Jan Siemens followed a call to Heidelberg in 2013). Other well-respected awards went to James Poulet (Paul Ehrlich and Ludwig Darmstaedter Prize for Young Researchers, 2012) and Klaus Rajewsky (José Carreras Award 2013, Medal of Honor of the Signal Transduction Society – STS) as well as to many other researchers at the MDC. You can read more about these awards in the annex of this research report.

das Zentren der Gesundheitsforschung initiiert hat und diese fördert. Das MDC beteiligt sich am DZHK sowohl über einen Sitz im Vorstand als auch mit wissenschaftlichen Projekten sowie mit der Administration der Fördermittel.

Wir haben in den letzten beiden Jahren auch den Weg dafür geebnet, dass das Berlin Institute for Medical Systems Biology (BIMSB) des MDC einen Standort in Berlin-Mitte erhalten wird. Es ist Bestandteil des Zukunftskonzeptes der Humboldt-Universität (HU) und hat damit zum Gewinn des Exzellenzstatus der HU beigetragen. Gemeinsam mit uns und der Charité hat die HU das Integrative Forschungsinstitut (IRI) für Lebenswissenschaften gegründet. Ziel des IRI für Lebenswissenschaften ist es, den Campus Nord der HU zu einem maßgebenden Wissenschaftspark im Herzen Berlins weiterzuentwickeln; das BIMSB wird dort voraussichtlich ab 2018 in einen Neubau ziehen.

Wir haben überdies im Jahr 2013 mit zwölf Partnerinstitutionen aus den Lebenswissenschaften das europäische Konsortium EU-Life gegründet. Es soll dem Informationsaustausch dienen, Karrierewege innerhalb Europas erleichtern und den Lebenswissenschaften gegenüber den Gremien und Organen der Europäischen Union mehr politisches Gewicht verleihen.

Basis all dieser wissenschaftspolitischen Erfolge und Entwicklungen ist die exzellente Arbeit unserer Wissenschaftlerinnen und Wissenschaftler. Im Jahr 2012 haben mehr als 30 internationale Gutachter das Max-Delbrück-Centrum evaluiert. Dabei schnitt unser Zentrum mit der Bestnote „Outstanding" ab. Dieser Erfolg unserer Arbeitsgruppen bildet sich auch ab in der Anzahl der Publikationen und in individuellen Preisen. So erhielt Nikolaus Rajewsky den Leibniz-Preis 2012, und seit der Veröffentlichung des letzten Research Reports haben wir zwei neue ERC Grants eingeworben. Damit liegt die Gesamtzahl dieser Projekte, die mit je mehreren Millionen vom Europäischen Forschungsrat ERC gefördert werden, am MDC bei zehn (ursprünglich waren es elf; der ERC-Grant-Gewinner Jan Siemens nahm 2013 einen Ruf nach Heidelberg an). Weitere ehrenvolle Preise gingen an James Poulet (Paul-Ehrlich- und Ludwig Darmstaedter-Nachwuchspreis, 2012) und Klaus Rajewsky (José Carreras

As a publicly funded research center, we are aware of our duty to ensure the sustainability of our work. This is why we place great importance on training young scientific talents including also school students. Currently, some 350 young women and men are working at the MDC to complete their doctoral theses. This takes place in the context of a structured training provision within the Helmholtz Graduate School "Molecular Cell Biology" and two other international exchange programs at the MDC. The training provision is structured into six fields of application: cardiovascular and metabolic diseases, cancer and immunology, neurobiology, developmental biology, medical systems biology and bioinformatics as well as structural biology. In training our doctoral candidates, we cooperate with universities and research institutions in Europe, Israel and the USA.

Yet our work in the field of providing education starts much earlier: by means of a nationwide much-noticed teacher education program ("Labor trifft Lehrer" – "Laboratory Meets Teacher"), we carry the working methods and results of our research in the field of molecular biology into schools. This is supported also by the Gläsernes Labor, which was initiated by the MDC and which will celebrate its 15th anniversary in 2014. With our activities we intend to contribute to a broader understanding of science in society, for our work is made possible by the tax payer's money. And today's school students are the ones who will continue our work as the researchers of tomorrow, thus further strengthening our knowledge-based society.

Wishing you informative reading,

Prof. Dr. Walter Rosenthal
Scientific Director of the MDC

Award 2013, Ehrenmedaille der Gesellschaft für Signaltransduktion) sowie an viele weitere Forscherinnen und Forscher des MDC. Sie können im Anhang dieses Forschungsberichts mehr zu den Preisen lesen.

Als öffentlich gefördertes Forschungszentrum sehen wir uns in der Pflicht, nachhaltig zu arbeiten. Daher legen wir großen Wert auf die Ausbildung des wissenschaftlichen Nachwuchses und auch von Schülerinnen und Schülern. Rund 350 junge Frauen und Männer sind derzeit am MDC, um eine Doktorarbeit zu machen. Dies geschieht innerhalb einer strukturierten Ausbildung durch die Helmholtz-Graduiertenschule „Molekulare Zellbiologie" und zwei weitere internationale Austauschprogramme am MDC. Die Ausbildung ist untergliedert in sechs Bereiche: Herz-Kreislauf- und Stoffwechselerkrankungen, Krebs und Immunologie, Neurobiologie, Entwicklungsbiologie, Medizinische Systembiologie und Bioinformatik sowie Strukturbiologie. Wir kooperieren bei der Doktorandenausbildung mit Universitäten und Forschungseinrichtungen in Europa, Israel und den USA.

Unsere Bildungsarbeit setzt aber noch früher an: Mit einem bundesweit viel beachteten Lehrerbildungsprogramm („Labor trifft Lehrer") bringen wir die Arbeitsweise und die Ergebnisse unserer molekularbiologischen Forschung in die Schulen. Dazu trägt auch das Gläserne Labor bei, das vom MDC initiiert wurde und im Jahr 2014 15 Jahre alt wird. Wir wollen mit unseren Aktivitäten zu einem breiteren Verständnis von Wissenschaft in der Gesellschaft beitragen, denn es sind die Steuerzahler, die unsere Arbeit ermöglichen. Und es sind die Schülerinnen und Schüler von heute, die morgen als Forscherinnen und Forscher unsere Arbeit fortführen und so die Wissensgesellschaft stärken werden.

Ich wünsche Ihnen eine informative Lektüre



Prof. Dr. Walter Rosenthal
Wissenschaftlicher Vorstand des MDC

Editorial Remarks

Redaktionelle Hinweise

This research report showcases the work of the Max Delbrück Center for Molecular Medicine (MDC) with a focus on the years 2012 and 2013. The MDC conducts biomedical basic research on the Campus Berlin-Buch within the framework of the Helmholtz Association, Germany's largest research organization. The Max Delbrück Center's aim is to transfer the findings as soon as possible into clinical applications with a focus on the diagnosis and therapy of diseases as well as on prevention. The MDC cooperates closely with the Charité – Universitätsmedizin Berlin, i.e. the joint university hospital and medical school of the Free University and the Humboldt University in Berlin. Both, Charité and MDC, are part of the Berlin Institute of Health (BIH) that was founded in 2013.

Research at the Max Delbrück Center is divided into four main areas: cardiovascular and metabolic diseases, cancer, diseases of the nervous system and systems biology. You will find this division reflected in this book which in its main parts contains portraits of individual research groups. As a fifth section we have included groups of the Experimental and Clinical Research Center (ECRC) which we run together with the Charité. All in all, you will find 70 research groups in this report.

We also provide excellent support to our scientists by high-throughput technology platforms for mass spectrometry, flow cytometry, confocal and two-photon microscopy, electron microscopy and magnetic resonance tomography, along with bioinformatics services and advanced data modeling. You will find brief descriptions of these technology platforms in this report, too.

Even though our work is focussed on basic biomedical research, we encourage our scientists to transfer their findings into business-models if possible. To facilitate technology transfer we have a program at the MDC called Pre-GoBio which provides

Dieser Forschungsbericht gibt einen Überblick über die Arbeit des Max-Delbrück-Centrums für Molekulare Medizin (MDC) der Jahre 2012 und 2013. Das MDC betreibt biomedizinische Grundlagenforschung auf dem Campus in Berlin-Buch und als Mitglied der Helmholtz-Gemeinschaft, Deutschlands größter Forschungsorganisation. Ziel der Arbeit am MDC ist es, die Ergebnisse der Grundlagenforschung möglichst rasch in die klinische Anwendung zu überführen, sei es in der Diagnose, der Therapie oder der Prävention von Krankheiten. Das MDC arbeitet dabei eng mit der Charité - Universitätsmedizin Berlin zusammen; letztere ist die gemeinsame medizinische Fakultät und Klinik der Freien Universität Berlin und der Humboldt-Universität zu Berlin. Beide, MDC und Charité, sind Teil des Berliner Instituts für Gesundheitsforschung/Berlin Institute of Health, das 2013 gegründet wurde.

Die Forschung am MDC ist in vier Bereiche gegliedert: Herz-Kreislauf- und Stoffwechselerkrankungen; Krebs; Erkrankungen des Nervensystems sowie medizinische Systembiologie. Diese Gliederung spiegelt sich im vorliegenden Bericht, der in seinem Hauptteil Porträts aller Arbeitsgruppen enthält. In einem fünften Bereich stellen sich die Gruppen des Experimental and Clinical Research Centers (ECRC) vor, das das MDC gemeinsam mit der Charité betreibt. Insgesamt finden Sie 70 Forschungsgruppen in diesem Bericht.

Das MDC bietet seinen Forscherinnen und Forschern exzellente Arbeitsbedingungen. Dazu gehört die Ausstattung mit Hochdurchsatz-Technologieplattformen für Massenspektrometrie, Durchflusszytometrie, konfokaler und 2-Photonen-Mikroskopie, Elektronenmikroskopie und Magnetresonanztomographie. Hinzu kommen Bioinformatik und mathematische Modellierung. Kurze Beschreibungen der MDC-Technologieplattformen befinden sich ebenfalls in diesem Bericht.



Photo: Guido Rottmann/MDC

MDC Campus in Berlin-Buch

initial support for scientists who then can apply for other programs within the Helmholtz Association or outside. We have dedicated a section of this research report to our technology transfer projects.

At the end of 2013, we hosted more than 350 PhD students in different programs. The part of the research report called Academics deals with our international networking programs, the Graduate School at the MDC, and offers overviews of awards that our scientists received and of the conferences in the past two years. Also included is a selection of our research projects that are realised through third-party funding.

In the Annex you will find, amongst other information, our outreach activities and the various affiliations or strategic partnerships of our institute, e.g. the Berlin Institute of Health, the German Centre for Cardiovascular Research "Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK)". EU-Life, and the Helmholtz Association. We also provide selected facts and figures that we hope you'll find useful.

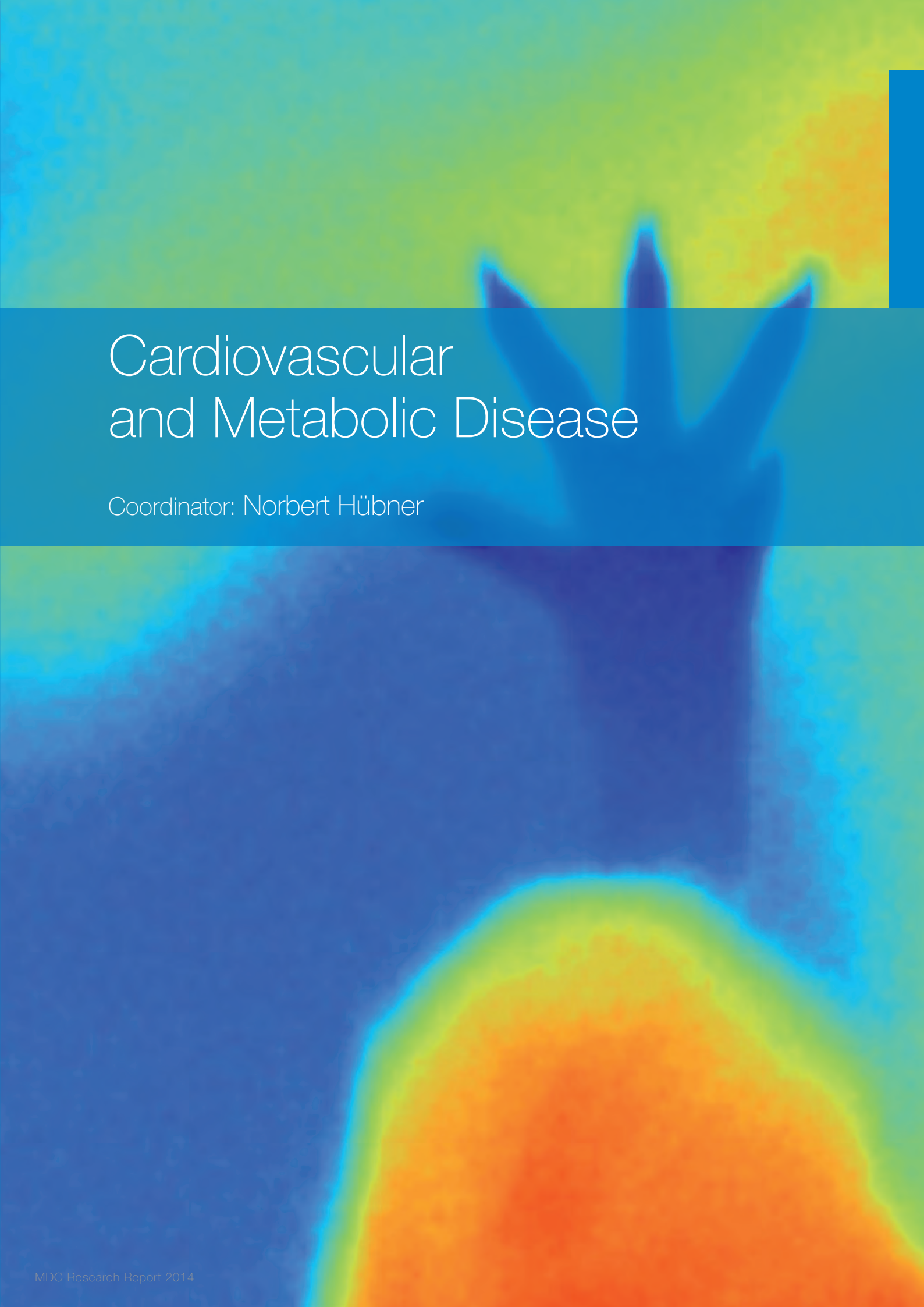
If you are missing any information in this report, please let us know. Our team will be happy to provide this information and we will see whether we can include additional sections in future reports. For any comments write to:
research_report@mdc-berlin.de

Zwar konzentriert sich die Arbeit am MDC auf Grundlagenforschung, aber wir ermutigen unsere Wissenschaftler dazu, ihre Ergebnisse in die Wirtschaft zu übertragen. Für diesen Technologietransfer haben wir das MDC-Programm „PreGoBio", das eine anfängliche Unterstützung für Forscherinnen und Forscher gewährt, die sich dann um andere Förderungen innerhalb der Helmholtz-Gemeinschaft oder darüber hinaus bewerben können. Einige dieser Technologietransferprojekte stellen wir in diesem Bericht vor.

Ende 2013 gab es mehr als 350 Studierende am MDC, die einen PhD-Abschluss anstreben. Der Berichtsteil mit dem Titel „Academics" enthält Beschreibungen unserer internationalen Programme innerhalb und außerhalb der Graduiertenschule sowie einen Überblick über Preise und Auszeichnungen unserer Forscherinnen und Forscher. Außerdem gibt es Listen unserer Konferenzen und Seminare sowie eine Auswahl von Forschungsprojekten, die mit Drittmitteln gefördert wurden.

Im Anhang finden Sie dann unter anderem Aktivitäten unserer Pressestelle und der Kommunikationsabteilung, Beschreibungen unserer Zugehörigkeiten und strategischen Partnerschaften, etwa Informationen zur Helmholtz-Gemeinschaft, zum Berliner Institut für Gesundheitsforschung/Berlin Institute of Health, dem Deutschen Zentrum für Herz-Kreislauf-Forschung (DZHK) und zu EU-Life. Ausgewählte Zahlen und Statistiken haben wir überdies für Sie zusammengestellt.

Wenn Sie Informationen in diesem Bericht vermissen, geben Sie uns bitte Bescheid. Unser Team wird sie gerne für Sie bereitstellen und wir werden prüfen, ob wir in künftigen Berichten weitere Kapitel anfügen. Ihre Fragen und Anmerkungen richten Sie bitte an:
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Cardiovascular and Metabolic Disease

Coordinator: Norbert Hübner

Cardiovascular and Metabolic Disease

Norbert Hübner

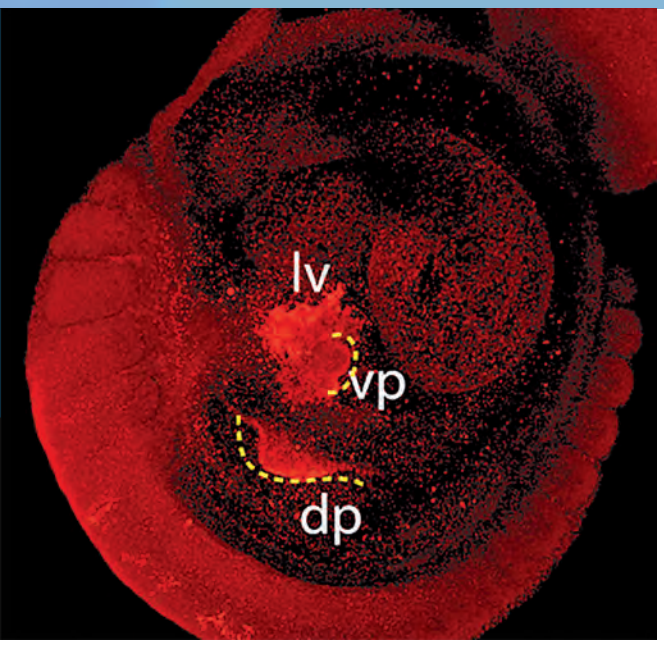
Cardiovascular and metabolic disease are the most common cause of death worldwide. Most cases of cardiovascular diseases are likely to be the result of contributions from multiple genes, and possibly complex epigenetic mechanisms that have yet to be discovered. Getting an experimental handle on these topics is a major challenge for the next decade. An increasing number of animal models – particularly strains of rats and mice – are available, yet novel models will be needed to convert findings into relevant clinical tools. Multifactorial diseases pose special problems including finding patients with a particular profile, extrapolating from a very small number of cases, and understanding complex interactions within cells. All of these challenges will require new types of modeling and a thorough integration of bioinformatics, clinical and experimental approaches.

The molecular analysis of cardiovascular and metabolic functions at the cellular level is a prerequisite for a mechanistic understanding of disease origins. It also provides a basis for the rational implementation of novel therapies to target cardiovascular and metabolic disorders. Projects in the program Fokus on key molecules and pathways involved in major processes of the cardiovascular and metabolic systems.

For example, the group of Michael Gotthardt studies the role of titin in the regulation of contractility in cardiac, skeletal, and smooth muscle. Specialized knock-in mouse models are used in conjunction with biomechanics and proteomics to define the functional roles of the many protein domains of titin that link mechanotransduction to energy homeostasis. Together with the Hübner lab he studied the splicing regulator of titin, the RNA-binding protein RBM20. This work emphasized the role of post-transcriptional regulation in the etiology of myocardial pathology. RBM20 interacts not only with titin but potentially in a network of 30 genes previously linked to cardiomyophathy, ion-homeostasis, and sarcomere biology.

Metabolic disturbances are emerging as major risk factors not only of cardiovascular but also neurodegenerative and oncological diseases. However, the molecular mechanisms that link the control of metabolism to other biological systems remain poorly understood. The group of Thomas Willnow is studying a novel group of cell surface receptors, the VPS10P domain receptors, which represent a unique class of signaling receptors with dual functions in the control of glucose and lipoprotein homeostasis in metabolic tissues and in the regulation of neuronal viability in the brain.

Organogenesis involves multiple sequential events such as fate specification, growth, differentiation, and morphogenesis, which ultimately result in the development of a functional mature organ. This step-wise process relies on a coordinated and highly complex interplay of signaling events and transcriptional networks. Importantly, aberrant developmental processes or a loss of their coordination are common causes of congenital defects of the cardiovasculature, liver, or pancreas. Learning to treat diabetes and various types of cardiovascular lesions will require a deeper knowledge of the genetic profile of myocardial or pancreatic progenitor cells. Deciphering the



Whole mount immunofluorescence staining with Prox1 antibody shows the liver (lv) and both dorsal (dp) and ventral (vp) pancreatic buds in a E9.5 mouse embryo.
adapted from Rodriguez-Seguel et al., Spagnoli, 2013

molecular signatures of "stem cell-ness" of particular cell types of the heart, liver, and pancreas, will allow us to isolate such cells for therapeutic purposes and possibly to instruct stem cells or progenitor cells to activate appropriate differentiation programs. Francesca Spagnoli is analyzing the transcriptome of hepatic and pancreatic progenitor cells through massive parallel sequencing isolated in vivo from mouse embryos before and after the initiation of organogenesis to reveal key regulatory genes that are differentially regulated in the two populations and are possibly involved in the pancreas- versus liver-fate decision. The group of Salim Seyfried studies lateral developmental processes in the zebrafish heart. They show that cardiac left-right asymmetry is regulated by Bmp, a growth factor of the TGF signaling pathway, and Nodal, and that Bmp signaling impairs the motility of cardiac progenitor cells.

Genetic variability among individuals plays an important role in the development and progression of cardiovascular disease. Genetic and genomic studies in human populations with chronic inflammatory disease are investigated by Young-Ae Lee to understand the etiology of chronic inflammation that is often shared by other forms of common human cardiovascular and metabolic diseases. In a collaborative systems biology approach, the groups of Nikolaus Rajewsky and Ferdinand LeNoble dicovered circular RNAs systematically and detected thousands of well-expressed, stable circRNAs, often showing tissue/developmental-stage-specific expression. Analysis revealed important regulatory functions for circRNAs and provides evidence that circRNAs form a large class of post-transcriptional regulators. Numerous circRNAs form by head-to-tail splicing of exons, suggesting a previously unrecognized regulatory potential of coding sequences.

The group of Matthew Poy is interested in microRNA regulation in metabolic states. They found that miR-184 is silenced in the pancreatic islets of insulin-resistant mouse models and type 2 diabetic human subjects. Their studies identify the targeting of Ago2 by miR-184 as an essential component of the compensatory response to regulate ß cell proliferation according to insulin sensitivity. They show that loss of Ago2 during insulin resistance blocked β cell growth and relieved the regulation of miR-375-targeted genes.

Understanding diseases at the molecular and cellular level is an important step in elucidating mechanisms that ultimately have their effects at the level of organs and organisms. But ultimately, the best model of human disease remains man. Following the idea of translational medicine the MDC cooperates with the Charité through the Experimental and Clinical Research Center (ECRC) encompassing novel imaging technology for whole-animals and man at the Ultrahigh-Field Magnetic Imaging Center (BUFF) run by Thoralf Niendorf pursuing projects including cardiac and metabolic imaging. Our translational activities will be strengthend in the future through the new Berlin Institute of Health (BIH) that was recently founded by MDC and Charité. In addition our program plays an eminent role in the German Center for Cardiovascular Research and the National Cohort for proband and patient oriented research in the prediction and prevention of cardiovascular and metabolic diseases.

The challenge remains to understand the regulatory genetic networks that influence cardiovascular and metabolic disease risk, to link molecular networks with physiological data, and to identify key regulatory nodes that can be targeted therapeutically to reduce the burden of cardiovascular and metabolic diseases in patients.



Photo: David Ausserhofer/MDC

Martin Falcke

Mathematical Cell Physiology

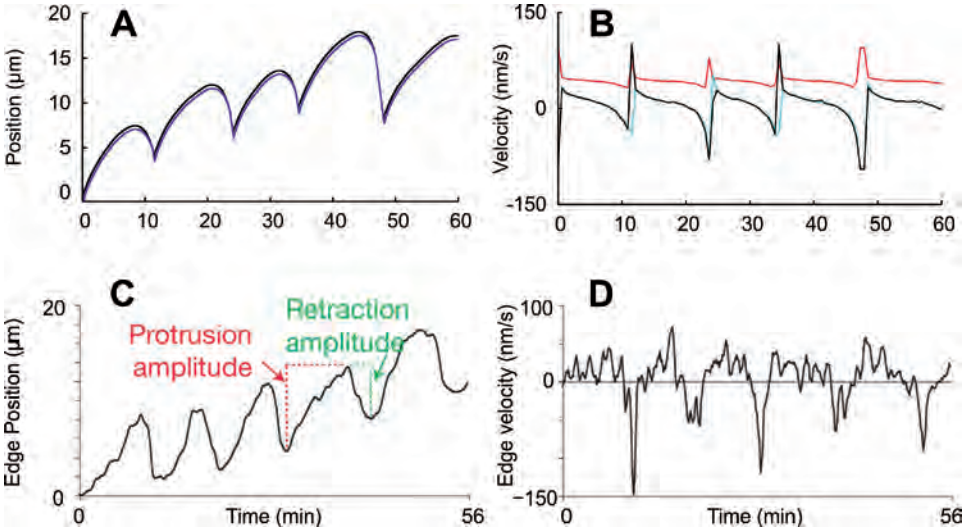
The group develops mathematical models for cell biology from single molecules, via protein complexes to cell level. Modelling serves to test and formulate biological hypotheses in a quantitative way as well as to predict the behavior of complex pathways and networks. Current projects comprise basic methods of stochastic modelling of cellular processes, cell morphodynamics, motility and protrusion generation, IP_3-induced Ca^{2+}-spiking and modelling of excitation contraction coupling in cardiac myocytes.

The project developing basic methods of stochastic modelling addresses both of the problems of state space explosion and verifiability of models. State space explosion is the exponential growth of the number of (microscopic) states of a stochastic system with the number of stochastic elements. Since each state requires a differential equation for its probability dynamics, that entails also the explosion of the mathematical effort to model these systems. Many equations entail large numbers of parameters, the values of which are usually not known in vivo and hence it is difficult to verify these models. On top of these technical difficulties, the states defined in this mathematical sense can usually not be identified experimentally. The concept we developed is based on observable states instead. It defines states rather by measurable function like 'closed' or 'open' than based on microscopic molecule states. Each functional state is a group of microscopic states. That entails mathematically a description by waiting time distribution functions instead of transition rates and a Master equation as system of integro-differential equations instead of differential equations. But the advantages outweigh these technical difficulties: The number of states stays small and the waiting time distributions can be directly measured. We have successfully applied these methods to IP_3-induced Ca^{2+} spiking and published the results (1, 2).

We develop mathematical models of cell morphodynamics in another line of research. Our model focusses in its current state on cells plated on a 2d substrate. Cell morphodynamics can then be recorded as the motion of the cell contour in a top down view on the cell. Two basic types of dynamics can be distinguished: dynamic generation and retraction of protrusions on the time scale of minutes (3) and the motion of the leading edge of existing protrusions on the time scale of tens of seconds (4). We are interested in the mechanisms causing these types of morphodynamics.

As a first step, we investigated the conditions for the existence of stable protrusions like lamellipodia. They form in mesenchymal cell motion and then guide the cell while moving on a 2d substrate, which may also be a membrane, tissue or tumor surface in an organism during cell motion in the context of development, metastasis or wound healing. Lamellipodia maintain their shape by the support of a network of actin filaments preferentially aligned around the direction of motion. Cross-linking molecules turn the ensemble of filaments into a stable network. New filaments are generated by branching off existing ones. Filament polymerization generates leading edge protrusion. Polymerization is terminated by proteins capping the polymerizing filament tip with a force dependent rate. We predicted this force dependence which has by now been experimentally confirmed. Binding of filament tips via linker molecules to the leading edge membrane may protect them from capping. Additionally, severing of filaments decreases the filament number.

We found (5) that no stable lamellipodium exists for low branching rates, because the



Comparison of simulated leading edge motion (A,B) (3) with experimental results (C,D) during repetitive protrusion generation of epithelial cells, Burnette et al., Nat. Cell Biol (2011). The black lines in B,D show leading edge velocity. The red line in B shows the network retrograde flow velocity, the light blue line the motion of the point where network stabilization by cross-linking is complete.

creation of new filaments by branching cannot compensate for filament extinction by capping and severing. The stable protrusion vanishes for small cross-linking rates, since the filaments grow long. That entails large severing rates and renders the filaments floppy, which increases the capping rate. The filament density decreases with increasing capping rate.

A larger external force decreases the capping rate via its force dependence. Furthermore, filaments shorten to adapt to the external force, which decreases the severing rate. Hence, applying an external force may cause protrusion formation.

This prominent role of external force motivated us to investigate how cell velocity depends on it. We explained the mechanism of the dynamic force-velocity relation recently (report 2012). Cells experience lasting forces in tissue and therefore, the stationary force-velocity relation is relevant there. This relation has not been measured, yet. We predict it to be distinctly different from the dynamic one, with larger velocity values, a single maximum for certain parameter ranges and a linear decrease of the velocity towards the stall force (5).

We were also interested in the mechanism and conditions for repetitive protrusion generation. In particular, we aimed at contributing to the discussion on the control of this process: Is the generation of each protrusion initiated by a signaling event upstream from nucleation promoting factors or is it self-organized by the actin polymerization machinery downstream from it. Our results demonstrate the possibility of the latter (3). In that model, protrusions are generated due to filament generation by branching. That generation is self-amplifying since the rate of branching is proportional to the number of existing filaments. In these cells, filaments then grow very long, become very floppy, susceptible to severing and capping, bend and form arcs. That terminates the existence of an individual protrusion. Our results agree very well with measurements including the highly non-trivial existence of sharp peaks in the leading edge and retrograde flow velocities. We could also reproduce the experimentally observed increase in period and amplitude of protrusion generation due to myosin inhibition (3). This excellent agreement between measurements and the mathematical model very much supports the idea of self-organized repetitive protrusion generation.

Selected Publications

1. K. Thurley, A. Skupin, R. Thul, M. Falcke, Fundamental properties of Ca^{2+} signals. Biochimica et Biophysica Acta (BBA) - General Subjects **1820**, 1185 (2012).

2. G. Moenke, M. Falcke, K. Thurley, Hierarchic stochastic modelling applied to intracellular Ca^{2+} signals. PLoS ONE **7**, e51178 (2012).

3. J. Zimmermann, M. Falcke, Formation of Transient Lamellipodia. PLoS one **9**, e87638 (2014).

4. A. Gholami, M. Enculescu, M. Falcke, Membrane waves driven by forces from actin filaments. NEW J PHYS **14**, 115002 (2012).

5. J. Zimmermann, M. Falcke, On the existence and strength of stable membrane protrusions. New Journal of Physics **15**, 015021 (2013).

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Photo: Uta Wrackmeyer/MDC

Michael Gotthardt

Neuromuscular and Cardiovascular Cell Biology

Our long-term goal is to establish how changes in biomechanics are translated into molecular signals and vice versa. Specifically, we are interested in understanding how increased filling of the cardiac ventricle leads to improved contraction (Frank-Starling mechanism of the heart), how alternative splicing relates to diastolic heart failure, and how exercise or immobilization change skeletal muscle growth. We focus on titin, the largest protein in the human body and the multifunctional coxsackie-adenovirus receptor (CAR). To lay the groundwork for the in vivo analysis of titin's multiple signaling, elastic, and adaptor domains, we have generated various titin deficient mice (knock-in and conditional knockout animals) and established a tissue culture system to study titin's muscle and non-muscle functions. We utilize a combination of cell-biological, biochemical, and genetic tools to establish titin as a stretch sensor converting mechanical into biochemical signals. Using a comparable loss of function approach we have created a conditional knockout of the coxsackie-adenovirus receptor to demonstrate that CAR is crucial for embryonic development and determines the electrical properties of the heart.

Cardiac alternative splicing

Michael Radke, Thirupugal Govindarajan, Martin Liss, Vita Dauksaite, Florian Hinze, Piotr Grabokwski

Alternative splicing is a major mechanism to adjust the cardiac proteome to the differential requirements of embryonic and postnatal physiology. We use a naturally occurring splice deficient rat strain, high throughput technology, and in silico analysis to identify factors that regulate cardiac alternative splicing and their target profiles. This approach has led us to identify various regulatory, metabolic, and structural proteins that determine cardiovascular physiology and adaptation. In addition to improving our knowledge of splicing as a basis for cardiac adaptation, we translate our findings towards developing novel therapeutic options for patients with cardiovascular disease emphasizing diastolic dysfunction with titin as a main contributor to the elastic properties of the heart. We have identified splice active compounds, which are currently tested in animal models of human disease as a prerequisite for future clinical studies.

Titin based mechanotransduction

Michael Radke, Thirupugal Govindarajan, Christopher Polack, Douaa Megahed

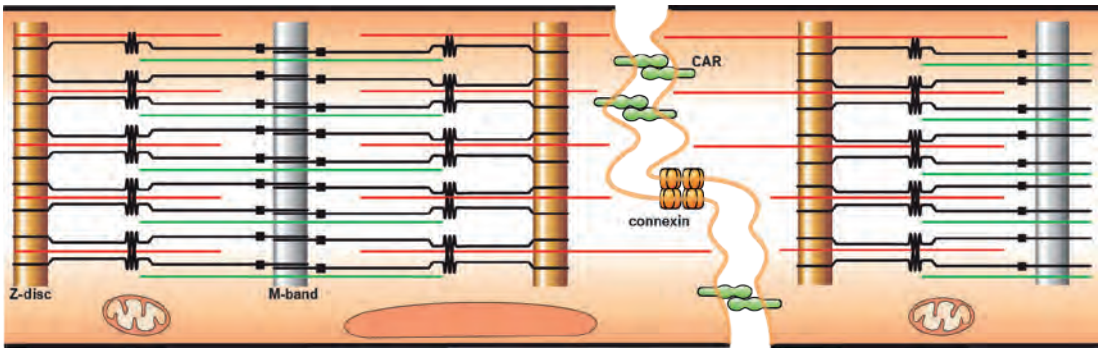
Titin is a unique molecule that contains elastic spring elements and a kinase domain, as well as multiple phosphorylation sites. Therefore, it has been frequently speculated that titin could act as a stretch sensor in muscle. So far it has remained unknown how the stretch signal is processed, i.e. how the mechanical stimulus stretch is converted into a biochemical signal.

To investigate the stretch signaling pathway, we apply mechanical strain in vivo (plaster cast for skeletal muscle; aortic banding for the heart) and in tissue culture (cultivation of primary cells on elastic membranes). The resulting changes in the proteome of our titin kinase and spring element deficient animals are used to map the mechanotransduction pathway.

Sarcomeric and non-sarcomeric functions of titin

Thirupugal Govindarajan, Franziska Rudolph, Claudia Fink, Joanna Kaldrack

Overlapping titin molecules form a continuous filament along the muscle fiber. Together with the multiple binding sites for sarco-



Schematic diagram of the mechanical and electrical connection between cardiomyocytes.
Titin (black) forms a continuous filament system along the muscle fiber. Its isoform expression is regulated by RBM20 to adapt the elastic properties of the myofilament. The proper transition of myofilaments between cells and their electrical coupling is regulated by the coxsackie and andenovirus receptor (CAR), which determines the localization of connexins and the attachment of filaments to the plasma membrane. Both titin and CAR have been linked to diverse cardiac diseases such as cardiomyopathy, diastolic dysfunction, and arrhythmia.

meric proteins, this makes titin a suitable blueprint for sarcomere assembly. The use of transgenic techniques does not only allow us to address the function of titin's individual domains in sarcomere assembly, but also to follow sarcomere assembly and disassembly using fluorescently tagged proteins. Understanding the structural and biomechanical functions of titin will help elucidate the pathomechanisms of various cardiovascular diseases and ultimately aid the development of suitable therapeutic strategies.
Recently titin has been proposed to perform non-muscle functions following its localization to mitotic chromosomes and cleavage furrows as well as the D-titin related chromosome abnormalities and aneupoidity found in Drosophila melanogaster. Our preliminary data indicate that titin is present in virtually every cell-type tested and our knockout of titin's M-band region displays a defect in implantation. Currently we are extending our analysis to the titin dependent regulation of the cell-cycle.

Functional analysis of the Coxsackie-Adenovirus Receptor

Uta Wrackmeyer, Joanna Kaldrack, Meghna Thakkar, Anne Merks

CAR was cloned as a receptor used by adeno- and coxsackievirus to enter cells but its physiological role has remained obscure. We have generated both tissue culture and animal models to study CAR's function in cardiac remodeling, inflammatory cardiomyopathy, and basic cellular processes such as endocytosis and cell-cell contact formation. Our data suggest a critical role of CAR in the conduction of electrical signals from the atria to the cardiac ventricle. The inducible heart-specific knockout of CAR has enabled us to completely block the entry of coxsackievirus into cardiomyocytes and prevent all signs of inflammatory cardiomyopathy. Developing inhibitors of CAR function, we aim to improve cardiac remodeling in myocardial disease.

Patents / Patent applications

Polynucleotides for use in medicine. Max-Delbrück-Centrum für Molekulare Medizin Berlin-Buch Dec, 29 2010: WO 2010/149332

Inducible site-directed mutagenesis through conditional gene rescue. Max-Delbrück-Centrum für Molekulare Medizin Berlin-Buch Oct, 21 2004: WO 2004/090131

LDL Receptor Signaling Pathways - US Patent Number 6,428,967. The University of Texas, 2001

Selected Publications

Marsman R.F.; Bezzina C.R.; Freiberg F.; Verkerk A.O.; Adriaens M.E.; Podliesna S.; Chen C.; Purfuerst B.; Spallek B.; Koopmann T.T.; Baczko I.; Dos Remedios C.G.; George A.L.; Bishopric N.H.; Lodder E.M.; de Bakker J.M.; Fischer R.; Coronel R.; Wilde A.A.; Gotthardt M.; Remme C.A. Coxsackie and adenovirus receptor (CAR) is a modifier of cardiac conduction and arrhythmia vulnerability in the setting of myocardial ischemia. Journal of the American College of Cardiology 18;63(6):549-59 (2014)

Guo W.; Schafer S.; Greaser M.L.; Radke M.H.; Liss M.; Govindarajan T.; Maatz H.; Schulz H.; Lincoln S.E.; Parrish A.M.; Dauksaite V.; Vakeel P.; Klaassen S.; Gerull B.; Thierfelder L.; Regitz-Zagrosek V.; Hacker T.A.; Saupe K.W.; Dec G.W.; Ellinor P.T.; MacRae C.A.; Spallek B.; Fischer R.; Perrot A.; Ozcelik C.; Saar K.; Hubner N.; Gotthardt M. RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. Nature Medicine 18 (5): 766-773 (2012)

da Silva Lopes K.; Pietas A.; Radke M.H.; Gotthardt M. Titin visualization in real time reveals an unexpected level of mobility within and between sarcomeres. Journal of Cell Biology 193 (4): 785-798 (2011)

Shi Y.; Chen C.; Lisewski U.; Wrackmeyer U.; Radke M.; Westermann D.; Sauter M.; Tschoepe C.; Poller W.; Klingel K.; Gotthardt M. Cardiac deletion of the coxsackievirus-adenovirus receptor abolishes coxsackievirus b3 infection and prevents myocarditis in vivo. Journal of the American College of Cardiology 53 (14): 1219-1226 (2009)

Lisewski U.; Shi Y.; Wrackmeyer U.; Fischer R.; Chen C.; Schirdewan A.; Juettner R.; Rathjen F.; Poller W.; Radke M.H.; Gotthardt M. The tight junction protein CAR regulates cardiac conduction and cell-cell communication. Journal of Experimental Medicine 205 (10): 2369-2379 (2008)

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Photo: David Ausserhofer/MDC

Ferdinand le Noble

Angiogenesis and Cardiovascular Pathology

Vascular network remodeling and the formation of new blood vessels – angiogenesis and arteriogenesis – play an important role in the pathophysiology of ischemic cardiovascular disease and cancer. My lab aims at generating novel genetic insights in the regulation of vascular development that can translate into therapeutic strategies. We focus on (1) differentiation and guidance of angiogenic vessels; (2) imprinting of arterial-venous identity in blood vessels by neural guidance genes and hemodynamic factors; (3) native collateral formation and vascular adaptation including arteriogenesis and angiogenesis in arterial occlusive diseases (stroke, heart infarct). We address vascular development using an integrative molecular and physiological approach in (transgenic) zebrafish, and mouse models. Our signalling pathways of interest include Dll4-Notch, VEGF-receptor-1, (post) transcriptional regulators of these pathways, and mechanosensing of hemodynamic factors, blood flow.

Tip Cell biology and Vessel Guidance

Recent studies of vascular network development in the embryo identified several novel aspects of angiogenesis crucial to generate a functional, and stable branched vascular network. These aspects include: the differentiation and guidance of endothelial tip cells in angiogenic vessels, and the formation of functional branches. We addressed tip cell formation and angiogenic sprout guidance using a genetic approach and in vivo imaging techniques in mouse, and zebrafish. We demonstrated that Delta-like 4 (Dll4) - Notch signalling plays a critical role in the differentiation of endothelial cells into tip cells in response to VEGF gradients. Recently, we defined the role of VEGF receptor-1, Flt1, in tip cell formation using zebrafish. We showed that soluble Flt1 produced by sprouting vessels acts, in a Notch-dependent manner, as a negative regulator of tip cell formation and sprout guidance. Soluble Flt1 mediated sprout guidance is a process that involved interaction with the nervous system. This interaction determines the distribution of soluble Flt1 surrounding the sprout, and controls the direction of sprout expansion. We are currently investigation the role of Flt1 at the neuro-vascular interface in the context of vessel guidance and neurogenesis.

Arteriogenesis and Ischemic Disease

Arteries form highly branched networks that ramify throughout the body. Most arteries branch into progressively smaller diameter arterioles that in turn branch into even smaller sized capillaries, which supply oxygen and nutriments to tissues and organs. However, some arterioles directly connect with other arterioles, usually from a neighboring arterial tree, and form so-called native collateral arteries. The native collateral circulation represents a major conduit for blood supply to tissues after occlusion of one of the feed arteries. Increases in shear stress and activation of inflammatory pathways upon occlusion trigger the outward remodeling of these pre-existing bypasses. Despite the clinical importance, factors influencing arterial branching morphogenesis and native collateral network

size remain poorly characterized. In the mouse brain, native arteriolar collateral networks appear as distal end-to-end arteriolar anastomoses. We recently found that the density of these collaterals is highest at time of birth, after which collateral density decreases with an increase of age. The Dll4-Notch signaling pathway is essential for embryonic vascular development and mediates specification of arterial identity and vessel sprouting. Using genetic loss and gain of function approaches in mice we found that Dll4-Notch signaling restricts pial collateral artery formation by modulating arterial branching morphogenesis during embryogenesis and adult $Dll4^{+/-}$ mice showed increased pial arterial collateral numbers. The Dll4-Notch pathway thus acts as a genetic determinant of native collateral number. The impact of these findings will be evaluated in experimental stroke models, and human stroke cohorts in collaboration with the Center for Stroke Research Berlin (CSB).

Hemodynamic forces exerted by flowing blood contribute to vascular branching morphogenesis during embryogenesis and trigger collateral arteriogenesis in the adult. Using special in vivo imaging techniques and systematic analysis of blood flow characteristics in developing vascular networks and collateral arteries we were able to identify a unique biophysical signal essential for triggering arterial identity gene expression, and outward remodeling. Expression profiling of flow activated collateral networks identified strong regulation of Gja5 and members of the bradykinin signaling pathway. Genetic ablation of Gja5 in mice resulted in reduced native collateral number and arteriogenesis. We find that bradykinin receptors act as positive modulators of arteriogenesis in mice and rats involving a contribution of peripheral immune cells, thereby linking hemodynamic conditions with inflammatory pathways.

Post-transcriptional regulators: miRNAs and sponges

Since the Dll4-Notch signaling pathway is evolutionarily highly conserved, we hypothesized the existence of microRNA families capable of targeting Dll4 and influencing vessel branching. The miR-30 family members are highly conserved between human, mouse, and zebrafish with perfect basepair matching with the 3'UTR of Dll4 mRNA in these species. Using loss and gain of function approaches in zebrafish, we found that endothelial miR-30a opposes Dll4 expression and accordingly promotes angiogenic cell behavior and arteriolar branching, in a Notch dependent manner. The influence of miR-30a on branching could be traced to an effect on tip-cell differentiation. We are currently evaluating whether miR-30 mimetic may be used to augment angiogenesis in ischemic settings. While miRNAs can directly regulate expression of most mRNAs in a diverse range of biological functions, surprisingly little is know about how and if mRNAs can escape regulation by a miRNA. A recently discovered mechanism for miRNA removal in a sequence specific manner is based on target sites acting as decoys or miRNA sponges. We recently identified a set of sponges in zebrafish and are currently exploring their impact on tissue morphogenesis and function.

Selected Publications

Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, **Le Noble F**, and Rajewsky N. (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495, 333-8.

Cristofaro B, Shi Y, Faria M, Suchting S, Leroyer AS, Trindade A, Duarte A, Zovein AC, Iruela-Arispe ML, Nih LR, Kubis N, Henrion D, Loufrani L, Todiras M, Schleifenbaum J, Gollasch M, Zhuang ZW, Simons M, Eichmann A, and **Le Noble F**. (2013). Dll4-Notch signaling determines the formation of native arterial collateral networks and arterial function in mouse ischemia models. Development 140, 1720-9.

Krueger, J., Liu, D., Scholz, K., Zimmer, A., Shi, Y., Klein, C., Siekmann, A., Schulte-Merker, S., Cudmore, M., Ahmed, A., and **Le Noble, F**. (2011). Flt1 acts as a negative regulator of tip cell formation and branching morphogenesis in the zebrafish embryo. Development 138, 2111-2120.

Klein, C., Mikutta, J., Krueger, J., Scholz, K., Brinkmann, J., Liu, D., Veerkamp, J., Siegel, D., Abdelilah-Seyfried, S., and **Le Noble, F**. (2011). Neuron navigator 3a regulates liver organogenesis during zebrafish embryogenesis. Development 138, 1935-1945.

Buschmann, I., Pries, A., Styp-Rekowska, B., Hillmeister, P., Loufrani, L., Henrion, D., Shi, Y., Duelsner, A., Hoefer, I., Gatzke, N., Wang, H., Lehmann, K., Ulm, L., Ritter, Z., Hauff, P., Hlushchuk, R., Djonov, V., van Veen, T., and **Le Noble, F**. (2010). Pulsatile shear and Gja5 modulate arterial identity and remodeling events during flow-driven arteriogenesis. Development 137, 2187-2196.

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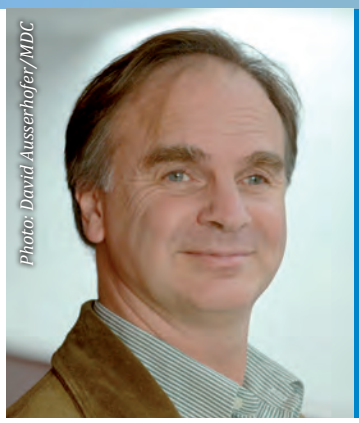


Photo: David Ausserhofer/MDC

Ingo Morano

Molecular Muscle Physiology

Contraction of all muscle types is elicited by increasing myoplasmic Ca^{2+} and interaction of Type II myosins with thin (actin) filaments of the sarcomere. In striated muscle, Ca^{2+} bind to troponin C, which turn the thin filament "on", allowing myosin actin interaction and force generation. We are studying the functional roles of subunits of key proteins of Ca^{2+} handling and force generation, i.e. the L-type Ca^{2+} channel and type II myosins in muscle. Any change of these proteins by mutation, differential gene expression, alternative splicing of the transcripts, post-translational modification, or sex modulate muscle function. Understanding muscle contraction regulation at the molecular and functional levels provides the opportunity to develop new therapeutic strategies for the treatment of cardiovascular and skeletal muscle dysfunction.

N- and C-terminal domains of essential myosin light chains (ELC) modulate cardiac functions

Janine Lossie, Clemens Köhnke, Petra Domaing

We modelled the missing 46 N-terminal amino acid of the ELC to the contemporary actin-myosin-S1 complex (Figure 1). The N-terminus of ELC showed a rod-like 91 Å structure being long enough to bridge the gap between the ELC core of myosin-S1 and the appropriate binding site of the ELC on the actin filament. ELC/actin interaction could be inhibited by a peptide-competition approach with synthetic peptides comprising the most N-terminal amino acids 1-15, which bind to the appropriate ELC binding site of actin. Recordings of cardiac function during peptide competition could then direct into a possible physiological role of ELC/actin interaction. To test the hypothesis that expression of N-terminal ELC peptides could modulate intrinsic contractility of the whole heart, we generated transgenic rats (TGR) that overexpressed minigenes encoding the N-terminal 15 amino acids of human atrial or ventricular ELC isoforms in cardiomyocytes. Systolic force generation, as well as the rates of both force generation and relaxation, rose in TGR lines that expressed the transgenic human ELC peptides.

We investigated structural properties and protein-protein interactions of a recombinant head-rod fragment of rat cardiac β-myosin heavy chain (MYH) and normal or five mutated (M149V, E143K, A57G, E56G, R154H)

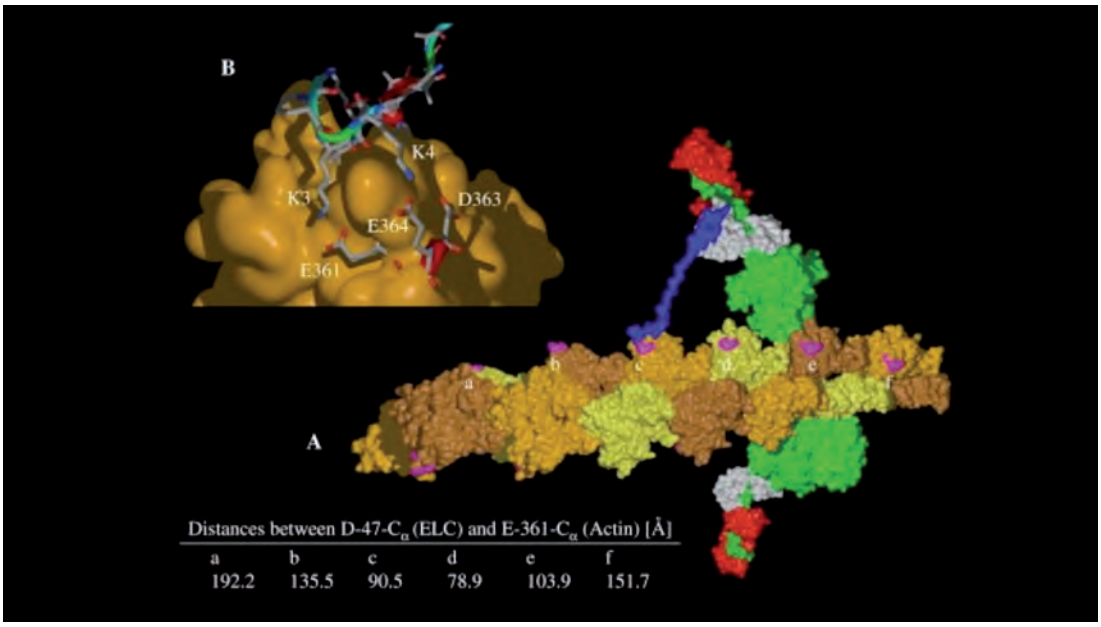


Figure 1. *3D-model of the actomyosin complex. (A) Gauss–Connolly surfaces are used to visualize the molecular complex. Actin units are coloured orange, brown, and yellow. The myosin S1 head (green), the regulatory light chain (red), the shortened essential light chain (white), the 46 N-terminal residues of A1 (blue), and clusters of acidic residues on actin (pink) are shown. (B) More detailed view on the potential interaction of N-terminal APKK of A1 with acidic residues on actin. Ionic interactions between lysine residues (K3 and K4) of APKK and acidic residues (E361 and E364) on actin were assumed.*

hVLC-1 forms. Affinity of M149V, E143K, A57G, and R154H mutated hVLC-1/MYH complexes were significantly lower compared with the normal hVLC-1/MYH complex interaction. In particular the E56G mutation induced an about 30fold lower MYH affinity. Disturbed myosin binding of mutated hVLC-1 may provide a pathomechanism for the development of hypertrophic cardiomyopathy.

We therefore hypothesized that hVLC-1 with weak myosin binding decreases myosin stiffness and deteriorates myosin and cardiac muscle functions. To test this hypothesis, we generated transgenic mouse strains expressing hVLC-1 (TgMhVLC-1) or hVLC-1E56G (TgME56G) in the heart. Echocardiographic analysis revealed no changes in cardiac dimension of TgME56G. Protein expression evaluated by SDS-PAGE and Western-blot analysis showed that the portion of hVLC-1 or hVLC-1E56G expression was 39.1% and 40.7%, respectively, of total VLC-1 in TgM myofibrils. Laser trap and in vitro motility assays showed that stiffness and actin sliding velocity of myosin prepared from TgME56G (1.67pN and 2.3µm/s, respectively) were significantly decreased compared with myosin from TgMhVLC-1 (1.25pN and 1.7µm/s, respectively). Furthermore, investigation with chemically skinned cardiac muscle fibers and isolated perfused hearts of TgM revealed that the isometric force generation of skinned fibers and left ventricular pressure development of perfused hearts from TgME56G (8.3mN/mm2 and 66.2mmHg, respectively) were significantly lower compared with TgMhVLC-1 (13.9mN/mm2 and 80.0mmHg, respectively). These novel findings provide valuable insights into the basal function of hVLC-1 in the heart and the development of familial hypertrophic cardiomyopathy relatedhVLC-1 mutations.

The functional role of the ahnak protein family in skeletal muscle fibers

Hannelore Haase, Frank Suhr Steffen Lutter

In the heart, ahnak1 is located at the sarcolemma and T-tubuli of cardiomyocytes indirectly associated with the voltage- dependent L-type Ca^{2+} channel (L-VDCC) via its β2-subunit (ß2) where it stimulates L-VDCC current. AHNAK functions and subcellular localization in skeletal muscle are unclear. Co-localization of ahnak1 and ahnak2 with vinculin clearly demonstrates that both proteins are components of the costameric network. In contrast, no ahnak expression was detected in the T-tubule system. A laser wounding assay with ahnak1-deficient fibers suggests that ahnak1 is not involved in membrane re-

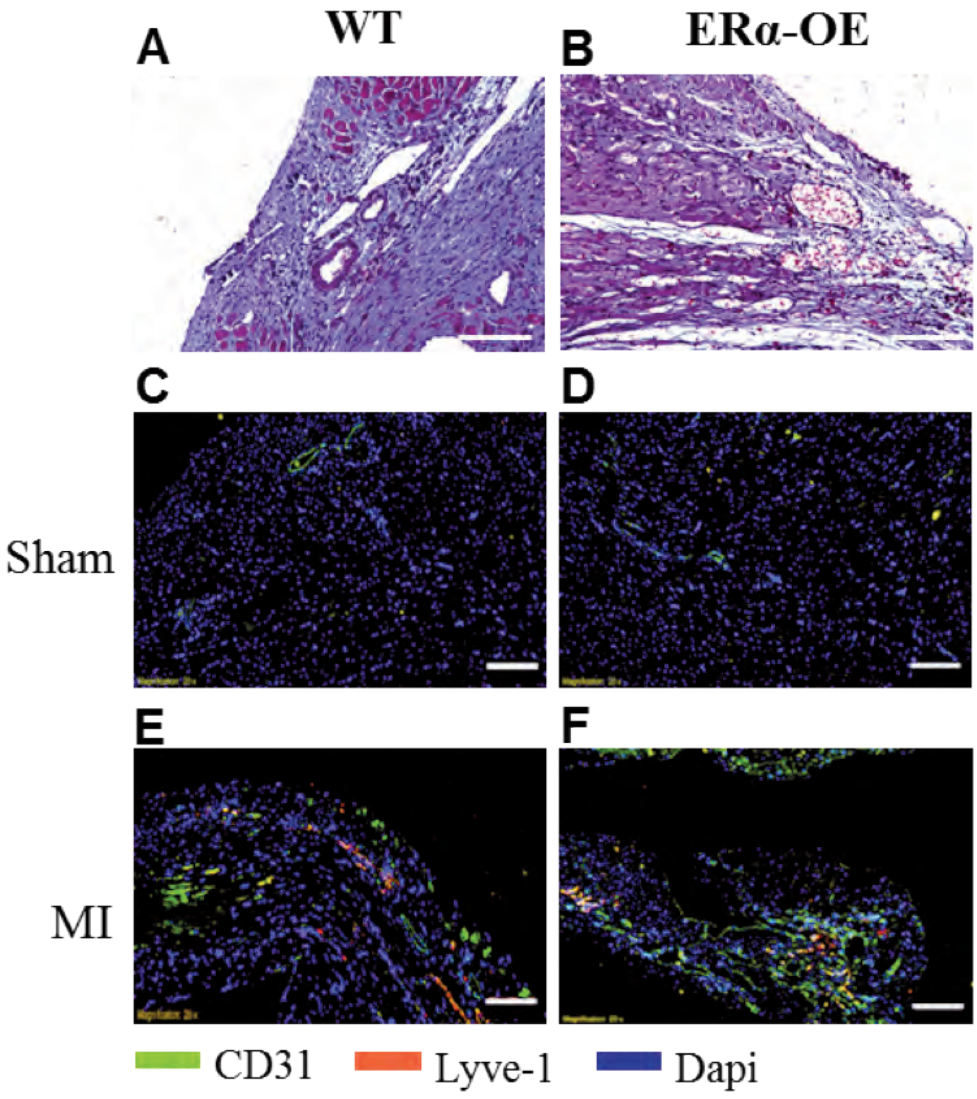


Figure 2. *ERα-overexpression in cardiomyocytes accelerates both angiogenesis and lymphangiogenesis predominantly in the peri-infarct areas in both sexes.* **A-B***: Representative images of H&E-stained paraffin sections of LV tissues from WT- and ERα-OE mice after myocardial infection (MI). ERα-OE mice hearts showed an increased occurrence of vascular-like structures in the peri-infarct area. 20x magnification, scale bar 100µm.* **C-F***: Representative immunofluorescence photographs of LV tissues 2 weeks after sham and MI operation from WT- (C* **&** *E) and ERα-OE (***D & F***) mice with CD31 and LYVE-1 staining (scale bar 100µm). For a better illustration of CD31 and LYVE-1 signals, higher magnification of the LV areas with strongest signals, i.e. in the peri-infarct area, have been shown. CD31-positive vessels (green), LYVE-1 positive vessels (red), nuclei (DAPI, blue).*

pair. Using atomic force microscopy (AFM), we observed a significantly higher transverse stiffness of ahnak1-/- fibers. These findings suggest novel functions of ahnak proteins in skeletal muscle. Interestingly, human muscle biopsy specimens obtained from patients with limb girdle muscular dystrophy showed that ahnak1 lost its sarcolemmal localization and appeared in the extracellular space in both muscular dystrophies.

Identification of adipocyte-derived cardiodepressant factor and their receptors

Christiane Woischwill, Karin Karczewski, in collaboration with Valeria Lamounier-Zepter, Dresden

The causal relationship between obesity and heart failure is broadly acknowledged; however, the pathophysiological mechanisms involved remain unclear. We observed that adipose tissue exerts highly potent activity with an acute depressant effect directly on cardiomyocytes, which may well contribute to increased heart failure risk in overweight patients. Through mass spectrometry and immunoblotting, we have identified this cardiodepressant factor as FABP4. FABP4 acutely depressed shortening amplitude as well as intracellular systolic peak Ca^{2+} in a dose-dependent manner in isolated rat cardiomyocytes.

Role of estrogen receptor alpha in sexually dimorphic cardioprotection against myocardial injury

Shokoufeh Mahmoodzadeh, Petra Sakel, in collaboration with Vera Regitz-Zagrosek, Berlin

Heart failure is associated with changes in myocardial architecture leading to cardiac dysfunction. Females have an improved heart failure prognosis and better survival. Animal studies showed that E2 protects the heart from ischemic injury. These data suggest cardioprotective effects of the female sex hormone, estrogen (17β-estradiol, E2). However, the mechanisms how E2 regulates myocardial functions have not yet been fully elucidated. E2 exerts its effects genomically and non-genomically by activating at least two E2 receptors, ERα and ERβ. To investigate the role of ERα in cardiomyocytes in the setting of myocardial ischemia, we recently generated transgenic mice with cardiomyocyte-specific overexpression of ERα and subjected them to myocardial infarction. Our study indicates that ERα protects female mouse cardiomyocytes from the sequelae of ischemia through induction of neovascularization (Figure 2) in a paracrine fashion and impaired fibrosis, which together may contribute to the attenuation of cardiac remodelling.

Selected Publications

Lättig-Tünnemann G, Prinz M, Hoffmann D, Behlke J, Palm-Apergi C, **Morano I**, Herce HD, Cardoso MC. (2011). Backbone rigidity and static presentation of guanidinium groups increases cellular uptake of arginine-rich cell-penetrating peptides. Nat. Commun 2:453.

Lamounier-Zepter V, Look C, Alvarez J, Christ T, Ravens U, Schunck W-H, Ehrhart-Bornstein M, Bornstein SR, **Morano I** (2009). Adipocyte fatty acid-binding protein suppresses cardiomyocyte contraction: a new link between obesity and heart disease. Circ. Res 105:326-34.

Aydt EM, Wolff G, **Morano I** (2007). Molecular modelling of the myosin-S1(A1) isoform. J Struct Biol 159:158-63.

Haase H, Dobbernack G, Tünnemann G, Karczewski P, Cardoso C, Petzhold D, Schlegel WP, Lutter S, Pierschalek P, Behlke J, **Morano I**. (2006). Minigenes encoding N-terminal domains of human cardiac myosin light chain-1 improve heart function of transgenic rats. FASEB J 20:865-73.

Mahmoodzadeh S, Leber J, Zhang X, Jaisser F, Messaoudi S, **Morano I**, Furth PA, Dworatzek E, Regitz-Zagrosek V. (2014). Cardiomyocyte-specific estrogen receptor alpha increases angiogenesis, lymphangiogenesis and reduces fibrosis in the female mouse heart post myocardial infarction. J Cell Sci Ther 2014, 5:1 (in press).

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Photo: Peter Himsel/MDC

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Electrochemical Signaling in Development and Disease

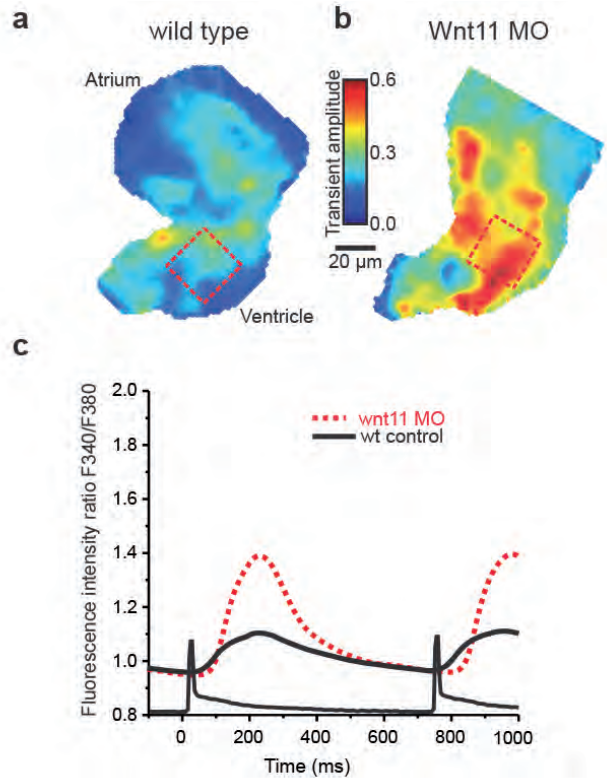
Physiological cues that include electrical and ionic impulses or metabolic intermediates have been long implicated in the orchestration of organ development during early embryonic patterning. While much is known about genetically encoded developmental pathways, the mechanisms by which the ions and small molecules interact with traditional morphogenetic signals are poorly understood. We use state-of-the-art technologies to explore the role of electrochemical and other physiologic stimuli during zebrafish embryogenesis. We focus on elucidating the interactions between calcium signaling and Wnt11 non-canonical signaling, a major developmental pathway regulating tissue morphogenesis, during cardiovascular development.

Molecular bases of interactions between Wnt non-canonical pathway and L-type Ca^{2+} channel

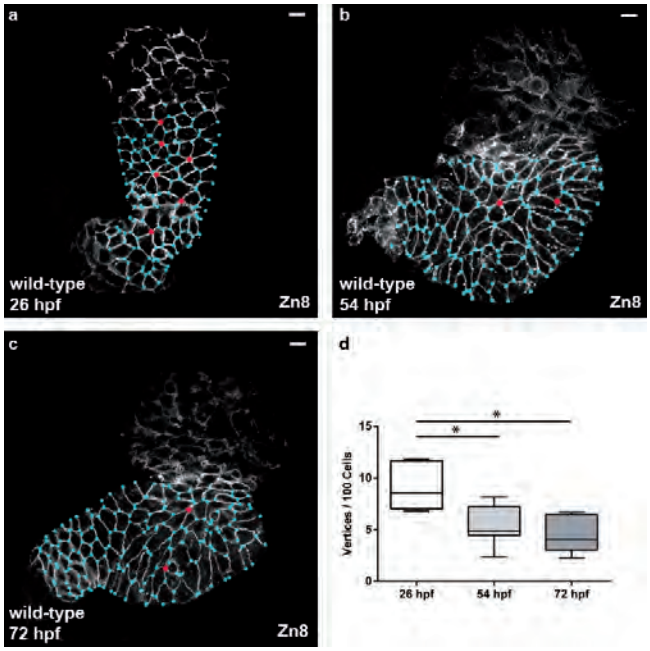
We have demonstrated that Wnt11 patterns intercellular electrical coupling in the myocardial epithelium through effects on transmembrane Ca^{2+} conductance mediated via the L-type calcium channel (LTCC). The cardiac LTCC belongs to a family of voltage-gated Ca^{2+} channels that are essentially the major point of entry for Ca^{2+} ions into the cytoplasm. Even though channel opening is primarily regulated by membrane potential, many other molecular mechanisms have been implicated in the modulation of LTCC function. Channel phosphorylation is the most commonly explored way of LTCC activation and Wnt signaling might be a plausible pathway to test for LTCC modulation via specific kinases like PKA, PKC or PKG. Next, Wnt signaling could interfere with LTCC localization to specific membrane microdomains, and thus modulate Ca^{2+} current through LTCC. Alternatively, Wnt signaling might interfere with LTCC channel trafficking. In order to define the molecular mechanism by which Wnt11 regulates LTCC at a subcellular resolution, we use cell-based models as well as zebrafish embryonic hearts.

Characterization of the components of the Wnt non-canonical transduction pathway involved in the L-type Ca^{2+} channel attenuation

The specificity and a wide range of diverse biological effects of Wnt signaling are mediated via binding and affinity of distinct Wnts to their corresponding receptors, Frizzleds. It has been shown that Wnt11 interacts with Fzd7 genetically as well as physically. A crucial step in Wnt signaling is the translocation of Dishevelled (Dsh) from the cytoplasm to the plasma membrane, relaying the Wnt signal to various downstream effectors. Dsh has been implicated so far in all known aspects of Wnt signaling, canonical and non-canonical. The N-terminal DIX domain regulates Wnt canonical signaling, while the central PDZ and C-terminal DEP domains of Dsh are responsible for the specific activation of the non-canonical, planar cell polarity (PCP) pathway. In addition, the DEP domain has been shown to have the ability to activate Wnt/Ca^{2+} signaling. It has been recently reported that a polybasic stretch within the DEP domain of Dsh is required for Dsh recruitment by Fzd to activate PCP signaling. We aim to define the requirement of the Wnt transduction machinery in the process of attenuation of L-type Ca^{2+} channel by



Wnt11 regulates Ca^{2+} transient amplitudes in cardiomyocytes: *a,b, Colour maps of Ca^{2+} transient amplitudes from wildtype hearts (a) and Wnt11 morphants (b). Colour code depicts Ca^{2+} transient amplitudes in fluorescent ratio units (F340/F380). Square indicate automated ROIs for measurements averaged in c. c, Averaged Ca^{2+} transients from ROIs in a,b.*



Cellular rearrangements drive cardiac chambers formation: *a,b,c, Vertices analysis in wildtype hearts stained with the membrane marker, zn8 at 26 hpf (a), at 54 hpf (b) and at 72 hpf (c). Blue dots mark 3-way vertices; red dots mark n-way vertices (n≥4). d, Graph showing the reduction of n-way vertices over time, between 26 hpf and 72 hpf. hpf=hours of post fertilization, scale bar=10µm.*

measuring the changes in intracellular Ca^{2+} fluxes in the series of Fzd and Dsh gain- and loss-of-function experiments.

Wnt non-canonical signaling patterns cardiac form

The profound morphogenetic changes that lead to the looped and chambered vertebrate heart occur concomitantly with the establishment and refinement of intercellular communication between cardiomyocytes. However, the molecular and cellular mechanisms that underlie the patterning of functional myocardial syncytium during early embryonic development are not thoroughly explored. We investigate how the two-chambered zebrafish heart forms from the linear heart tube. Although cardiac muscle cells are of mesodermal origin, detailed studies demonstrated that embryonic zebrafish myocardium retains its epithelial characteristics. Thus, we hypothesize that the mechanisms that drive epithelial tissue remodeling might play an important role during cardiac chamber formation. We were able to show that cardiomyocyte rearrange within the myocardial epithelium to form two chambers. We are currently investigating how Wnt non-canonical pathway guides the junctional remodelling and the cellular rearrangements during this process.

Selected Publications

Becker, J.R., Chatterjee S., Robinson T.Y., Bennett J.S., **Panáková D.**, Galindo C.L., Zhong L., Shin J.T., Coy S.M., Kelly A.E., Roden D.M., Lim C.C., and MacRae C.A. (2014). Differential activation of natriuretic peptide receptors modulate cardiomyocyte proliferation during development. Development 141, 335-45.

Wang, J.H., **Panáková, D.**, Kikuchi, K., Holdway, J.E., Gemberling, M., Burris, J.S., Singh, S.P., Dickson, A.L., Lin, Y.F., Sabeh, M.K., Werdich, A.A., Yelon, D., Macrae, C.A., and Poss, K.D. (2011). The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. Development 138, 3421-3430.

Becker, J.R., Deo, R.C., Werdich, A.A., **Panáková, D.**, Coy, S., and MacRae, C.A. (2011). Human cardiomyopathy mutations induce myocyte hyperplasia and activate hypertrophic pathways during cardiogenesis in zebrafish. Disease Models & Mechanisms 4, 400-410.

Panáková, D.*, Werdich, A.A.*, and MacRae, C.A. (2010). Wnt11 patterns a myocardial electrical gradient through regulation of the L-type Ca(2+) channel. Nature 466, 874-878.

Panáková, D. *, Sprong H. *, Marois E., Thiele C., and Eaton, S. (2005). Lipoprotein particles are required for Hedgehog and Wingless signalling. Nature 435, 58-65.

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Anchored Signalling

Vasopressin-mediated water reabsorption

Antidiuretic hormone (Arginin-vasopressin, AVP) fine tunes body water homeostasis. Its binding to vasopressin V2 receptors on the surface of renal collecting duct principal cells leads to a raise of the level of the second messenger cAMP, which activates protein kinase A (PKA). PKA phosphorylates the water channel aquaporin-2 (AQP2) and thereby induces its redistribution from intracellular storage vesicles into the plasma membrane. The membrane insertion of AQP2 increases the water permeability of the collecting duct, facilitates water reabsorption from primary urine and through this fine-tunes water homeostasis (Fig. 1).

Diabetes insipidus is associated with a massive loss of hypotonic urine and polydipsia (massive thirst). Loss of function mutations in the vasopressin V2 receptor or AQP2 genes cause congenital nephrogenic diabetes insipidus (NDI), lithium treatment of depression leads to acquired nephrogenic diabetes insipidus; Elevated levels of AVP with excessive water retention can be found in heart failure, liver cirrhosis or the syndrome of inappropriate antidiuretic hormone secretion (SIADH).

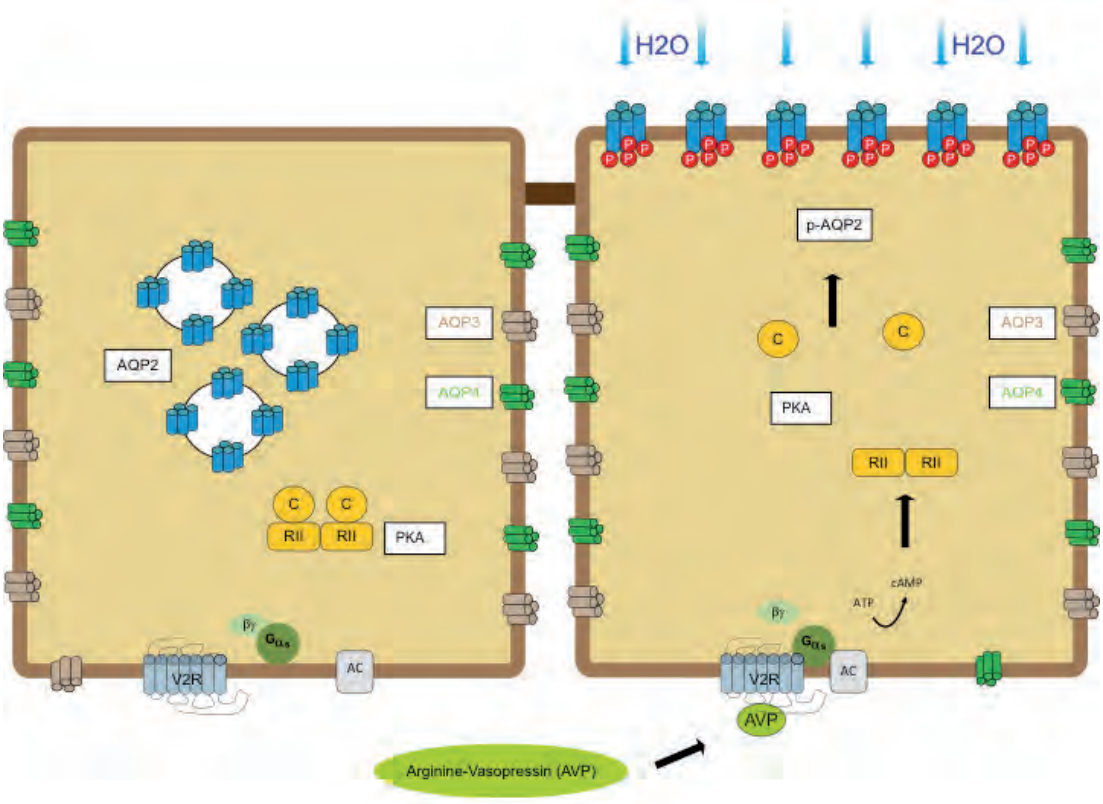


***Fig. 1**. Model of vasopressin (AVP)-mediated water reabsorption in renal collecting duct principal cells.*

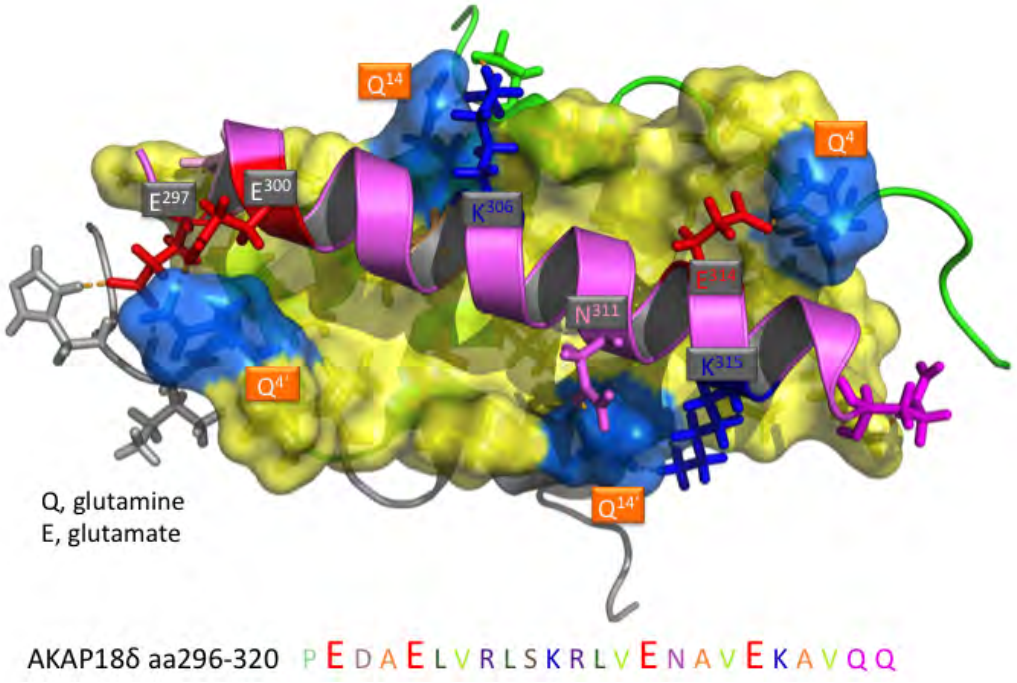


Fig. 2. *In silico model of the interaction of the D/D domain of RIIα subunits of PKA with the AKAP18δ-derived peptide AKAP18δ-L314E.*

A major focus of our group is to gain mechanistic insight into the functioning AVP-mediated water reabsorption through identification of novel proteins and their interacting partners that participate in the control of AQP2. The redistribution of AQP2 resembles cAMP-induced exocytic processes. Thus the elucidation of molecular mechanisms underlying the control of AQP2 will not only help to understand this but also other physiologically highly relevant cAMP-dependent exocytic processes such as renin secretion from juxtaglomerular cells or proton secretion from gastric parietal cells. We have previously identified several proteins and their interacting partners participating in the control of AQP2. These include A-kinase anchoring proteins (AKAPs), the GTP-binding proteins RhoA and Rab11, the cytoskeletal components F-actin and myosin and the motor proteins myosin Vb and dynein, which mediate intracellular trafficking of AQP2-bearing vesicles along the cytoskeleton. Although the identification of these and several other proteins has shed some light on mechanisms controlling AQP2, the precise molecular mechanisms underlying the regulation of AVP-mediated water reabsorption are largely unclear.

A second major focus of the group is the development of novel strategies for pharmacological interference with AVP-mediated water reabsorption in order to find new ways for the treatment of cardiovascular diseases characterised by excessive AVP-dependent water reabsorption, in particular heart failure.

Small molecules as tools for the elucidation of the molecular mechanisms underlying the control of AQP2

We pursued a small molecule screening as an unbiased approach for the identification of novel proteins involved in controlling AQP2. In cooperation with the screening unit at the Leibniz Institute for Molecular Pharmacology (FMP), we screened a library of 17,700 small molecules for inhibitors of the cAMP-induced redistribution of AQP2 utilizing mouse collecting duct-derived MCD4 cells as model system and automated immunofluorescence microscopy. This approach yielded 17 inhibitors; amongst them 4-acetyldiphyllin (4AD), a selective blocker of vacuolar H^+-ATPase. 4AD increased pH levels of intracellular vesicles and caused an accumulation of AQP2 in the Golgi. The compound prevented the cAMP-dependent and PKA-catalysed phosphorylation of AQP2 at serine 256, the key trigger for its redistribution into the plasma membrane. 4AD did not affect cAMP levels, PKA activity or cAMP-induced phosphorylations at other sites of AQP2. Thus our phenotypic cell-based screening approach showed functional vacuolar-H^+-ATPase is required for the cAMP-induced phosphorylation of AQP2 at S256 and its redistribution to the plasma membrane but is not required for phosphorylation of AQP2 at other residues. We have identified additional small molecule inhibitors of the redistribution of AQP2. In the future, we aim to identify their targets and modes of action. The identification of the targets is expected to reveal fur-

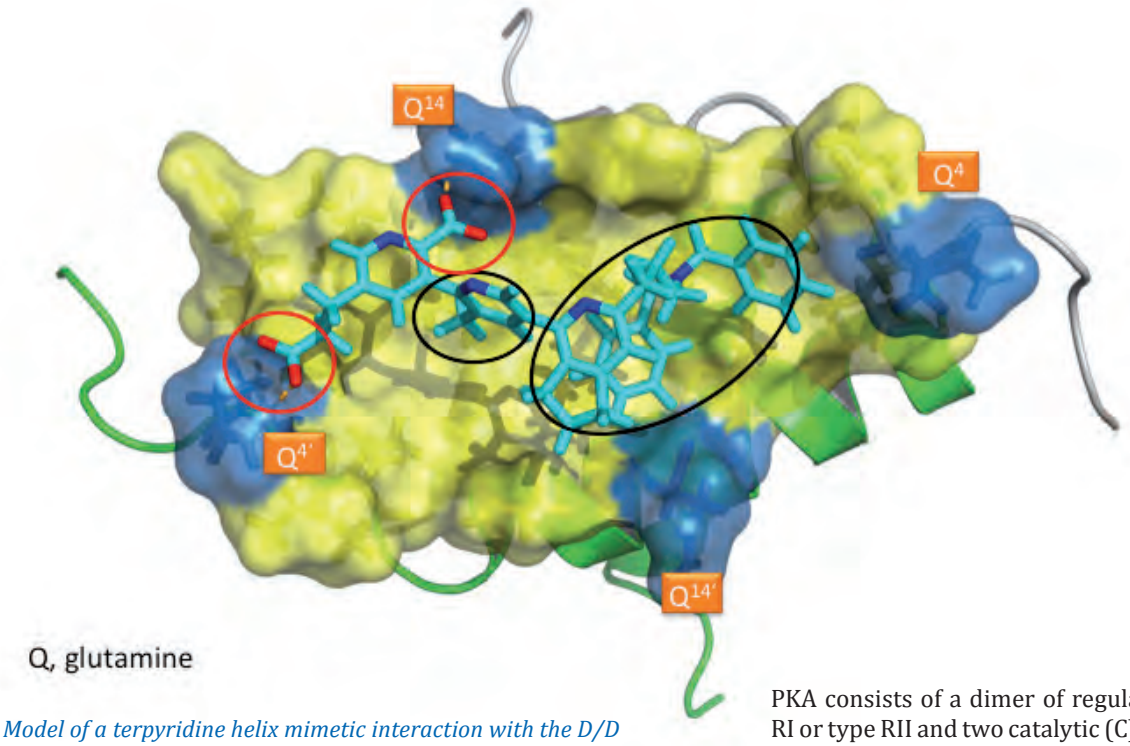


Fig. 3. *Model of a terpyridine helix mimetic interaction with the D/D domain of RIIα subunits. The red rings indicate carboxyl groups of the first pyridine that are likely to interact with Q4' and Q14 of the two protomers of the D/D dimer. The black ellipses indicate the ortho methyl group of the second pyridine, the condensed cyclopentyl ring of the third pyridine and the following two benzyl rings of the terpyridine molecule, which mimic the hydrophobic phase of the AKAP18δ-L314E helix and interact with the hydrophobic bottom of the binding pocket.*

ther insight into the mechanisms controlling the trafficking of AQP2. Moreover, the molecules may serve as starting points for the development of drug candidates for the treatment of cardiovascular diseases characterised by excessive AVP-mediated water reabsorption, such as heart failure.

Pharmacological interference with AKAP-dependent protein-protein interactions – searching novel strategies for the treatment of heart failure

Cells respond to a certain stimulus in a highly specific manner. A fundamental principle underlying this specificity is the tethering of components of signalling cascades to defined cellular compartments, i.e. to their sites of action. AKAPs are a family of around 50 scaffolding proteins that fulfil this task. Their unifying property is their ability to directly bind PKA. However, they all bind additional signalling proteins such as other kinases, phosphatases or phosphodiesterases via direct protein-protein interactions. Thereby, they form signalling hubs to coordinate cellular signalling spatially and temporally.

PKA consists of a dimer of regulatory type RI or type RII and two catalytic (C) subunits. Binding of cAMP to the R subunits induces release of the C subunits that then phosphorylate their substrates in close proximity. The N-terminal dimerization/docking (D/D) domains of the R subunits (amino acids 1-44) mediate dimerization and form a hydrophobic pocket that directly interacts with a conserved 14–25 amino acids long α-helix of AKAPs (RII-binding domain). Synthetic peptides, derived from the PKA-binding domain of AKAPs disrupt AKAP-PKA interactions by competitive binding to the D/D domain of R subunits. We derived such PKA disruptor peptides from the PKA binding domain of AKAP18δ, which we had previously identified. One of the peptides, AKAP18δ-L314E binds PKA RII subunits with subnanomolar affinity, e.g. to RIIα subunits with K_D = 0.4 nM.

We observed that inhibition of AKAP-PKA interactions in primary inner medullary collecting duct (IMCD) cells with membrane-permeable versions of peptide AKAP18δ-L314E prevents the AVP-induced insertion of AQP2 into the plasma membrane, presumably because the peptides displace PKA from its close proximity to AQP2 and thereby prevent the PKA phosphorylation of AQP2 at S256. Other groups as well as ours showed that disruption of AKAP-PKA interactions in cardiac myocytes and whole rat hearts increases contractility. Since inhibition of AVP-mediated water retention and improvement of cardiac contractility should relieve symptoms of heart failure, inhibition of AKAP-PKA interactions in renal principal cells and cardiac myocytes may pave the way to a new concept for the treatment of heart failure.

Terpyridine helix mimetics targeting AKAP-PKA interactions

Although PKA anchoring disruptor peptides are invaluable molecular tools for studying functions of AKAP-PKA interactions, the generally poor oral availability and stability of peptides limits their use in animal studies and their potential as drugs. Small molecules and non-peptide helix mimetics are considered as alternatives to peptides. We identified several small molecule disruptors of AKAP-PKA interactions by high-throughput screening and are currently validating and optimising these molecules.

Non-peptide helix mimetics including terpyridine scaffolds mimic α-helixes by projecting side chains of amino acids from a rod-like axis. Such mimetics are designed to combine the specificity/target selectivity of peptides with the stability and membrane permeability of small molecules. Based on our peptide AKAP18δ-L314E we developed pyridines as helix mimetics, taking advantage of the favorable solubility, conformational orientation, and biological activity of helix mimetics of this class. Starting point was an interaction model in which hydrophobic residues (L301, L304, L308) of the peptide interact with the hydrophobic bottom of the D/D domain of RIIα subunits and where the hydrophilic side chains of the peptide interact with hydrophilic residues such as Q4 at the peripheral rim of the pocket formed by the D/D dimer (Fig. 2). Fig. 3 shows one of the mimetics docked into the D/D domain of RIIα subunits. The carboxyl groups of the first pyridine address hydrophilic residues at the rim of the hydrophobic pocket and mimic, for example, the side chain of E300 of the peptide that is likely to interact with Q4. The ortho methyl group of the second pyridine, the condensed cyclopentyl ring of the third pyridine and the following two benzyl rings of the molecule mimic the hydrophobic phase of the AKAP18δ-L314E helix and interact with the hydrophobic core (Fig. 2 and 3) of the binding pocket. These new helix mimetics interfere with AKAP-PKA interactions in vitro and in cultured cells. Therefore, the terpyridine molecules represent the first biologically active and non-peptidic compounds that block the D/D domain of regulatory subunits of PKA and thereby interfere with AKAP-PKA interactions.

In the future, we aim to develop our small molecules and helix mimetics towards drug candidates for the treatment of heart failure.

Patents / Patent applications

Klußmann, E., Tröger, J., Rosenthal, W. AKAP-Lbc-Hemmstoffe zur Therapie der kardialen Hypertrophie. EP12174673.9.

Selected Publications

Schäfer G, Milić J, Eldahshan A, Götz F, Zühlke K, Schillinger C, Kreuchwig A, Elkins JM, Abdul Azeez KR, Oder A, Moutty MC, Masada N, Beerbaum M, Schlegel B, Niquet S, Schmieder P, Krause G, von Kries JP, Cooper DM, Knapp S, Rademann J, Rosenthal W, Klussmann E. Highly Functionalized Terpyridines as Competitive Inhibitors of AKAP-PKA Interactions. Angew. Chem. Int. Ed. 52:12187-12191, 2013

Bogum J, Faust D, Zühlke K, Eichhorst J, Moutty MC, Furkert J, Eldahshan A, Neuenschwander M, von Kries JP, Wiesner B, Trimpert C, Deen PM, Valenti G, Rosenthal W, Klussmann E. Small-molecule screening identifies modulators of aquaporin-2 trafficking. J. Am. Soc. Nephrol. 24:744-58, 2013

Christian, F., Szaszák, M., Drewianka, S., Friedl, S., Lorenz, D., Furkert, J., Vargas, C., Schmieder, P., Götz, F., Göttert, H., Joshi, M., Reif, B., Haase, H., Morano, I., Kass, R., Hampel, K., Kashin, D., Genieser, H.-G., Herberg, F. W., Willoughby, D., Cooper, D. M. F., Baillie, G. S., Houslay, M. D., Kries, J. P., Zimmermann, B., Rosenthal, W. and Klussmann, E. Small molecule AKAP/PKA interaction disruptors that activate PKA increase cardiac contractility. J. Biol. Chem. 286, 9079–9096, 2011.

Nedvetsky, P.I., Tabor, V., Tamma, G., Beulshausen, S., Mutig, K., Boltzen, M., Petrucci, O., Vossenkämper, A., Wiesner, B., Bachmann, S., Rosenthal, W., Klussmann, E. Reciprocal regulation of aquaporin-2 abundance and degradation by protein kinase A and p38-MAP kinase. J. Am. Soc. Nephrol. 21:1645-56, 2010 Recommended for reading by Faculty of 1000

Hundsrucker, C., Skroblin, P., Christian, F., Zenn, M., Popara, V., Joshi, M., Herberg, F. W., Reif, B., Rosenthal, W. and Klussmann, E. The domain of unknown function (DUF) 727 of glycogen synthase kinase interaction protein (GSKIP) facilitates anchoring of protein kinase A. J. Biol. Chem. 285, 5507–5521, 2010.

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Kai Schmidt-Ott

Experimental and Translational Kidney Research

The kidney is a central organ of cardiovascular homeostasis. It excretes toxins into the urine, regulates volume status and solute concentrations in the body, and produces hormones. Chronic kidney disease constitutes an important risk factor for many other diseases, including cardiovascular diseases and cancer. The kidney is composed of structural units called nephrons, which consist of several different types of renal epithelial cells that facilitate directional transport. Our group studies the molecular mechanisms of epithelial morphogenesis during embryonic development and the maintenance of epithelial integrity later in life. We use experimental model systems, including cell and organ culture techniques and animal models, and carry out clinical studies to carry our findings from bench to bedside.

From kidney development to kidney disease

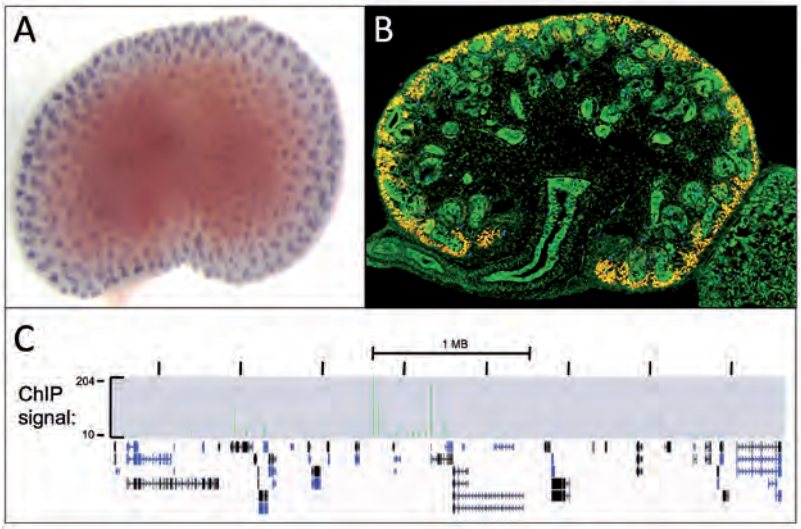
During embryonic development, formation of the definitive kidney is initiated, when the ureteric bud, an epithelial tubule extending from the posterior Wolffian duct, interacts with an adjacent progenitor cell population, the metanephric mesenchyme. The Wolffian duct undergoes branching morphogenesis to give rise to the ureter, renal pelvis and collecting duct system, while the metanephric mesenchyme converts into epithelial cells that subsequently get patterned along the proximal-distal axis to form the different cell types of the nephron. As these cells differentiate, they obtain epithelial characteristics, including the establishment of apico-basal polarity and the formation of epithelial-specific junctions. Mammalian embryonic kidney development constitutes a classical model system in developmental biology, in which branching morphogenesis and tubulogenesis occur in parallel. The sequence of events can be closely monitored in developing mice or in organ cultures, in which key in vivo aspects of nephrogenesis are recapitulated. Exogenous or genetic perturbations of kidney development result in congenital kidney diseases. Furthermore, adult kidney epithelia preserve the ability to reactivate molecular pathways from earlier developmental stages in certain disease states, including tumors, kidney fibrosis, and acute tubular injury. Hence, a detailed understanding of these molecular mechanisms will help to improve the molecular diagnosis of kidney diseases and provide novel targets for therapy.

Transcription factors in epithelial development and homeostasis

We study the role of transcription factors in cellular differentiation and morphogenesis, and in regeneration and tumorigenesis. We focus on transcription factos from the TCF/Lef family and from the grainyhead family, which we and others found to govern critical aspects of epithelial morphogenesis and differentiation, and in healing of injured epithelial structures. For instance, two members of the grainyhead family, called Tcfcp2l1 and Grhl2, are highly expressed by nephron epithelia during development and in adulthood. In ongoing studies, we are analyzing the roles of these transcription factors in development and disease. The projects benefit from our close collaborations with the groups of Jonathan Barasch (Columbia University), Frank Costantini (Columbia University, New York), Carmen Birchmeier (MDC), Thomas Willnow (MDC), and Michael Bader (MDC).

Systems biology of kidney diseases

Kidney injury and phenotpyical transformations of kidney epithelia in congenital and neoplastic kidney diseases are associated with



Developing kidneys are analyzed by whole-mount in situ hybridization (A) or immunofluorescent staining (B). (C) shows an example of an experiment to detect genome-wide binding of a transcription factor to DNA as determined by chromatin immunoprecipitation followed by next generation sequencing. Binding intensity (green) is shown relative to the genomic position. Genes (blue) are shown on the bottom.

widespread alterations of the genome and its expression. We are combining next generation sequencing based techniques and bioinformatics to map alterations of the genome, epigenome, and transcription factor binding sites in normal and diseased kidney cells. For this purpose we are collaborating with the Department of Urology at Charité (Kurt Miller), with the group of Walter Birchmeier (MDC), and with systems biology groups at the Berlin Institute of Medical Systems Biology (BIMSB). We believe that a detailed elucidation of the genome-wide alterations in diseased renal cells will yield an unprecedented opportunity to understand in detail the molecular mechanisms of renal disease.

Molecular diagnosis of renal injury

Re-expression of embryonic marker molecules is a common feature in disease states and is believed to participate in compensation and regeneration. Neutrophil gelatinase-associated lipocalin (NGAL) is a protein in the developing kidney that is sufficient to induce differentiation in embryonic renal epithelial progenitors. NGAL is also markedly reactivated in tubular injury of the kidney and its urinary excretion is closely correlated with the temporal onset and severity of tubular injury. In collaboration with Jonathan Barasch (Columbia University, New York), Klemens Budde (Department of Nephrology, Charité), Friedrich C. Luft (ECRC), Ralph Kettritz (ECRC), we are conducting basic and clinical studies to understand the biology and clinical applications of NGAL in kidney injury. We are aiming to optimize diagnostic algorithms that utilize NGAL measurements to predict renal injury and to differentiate renal injury from related clinical entities. We also aim to identify additional novel and phase-specific biomarkers of kidney injury and compare them to the available diagnostic methods in clinical medicine.

Acknowledgements

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For additional information please visit: http://www.mdc-berlin.de/schmidt-ott

Selected Patents

Compositions for modulating growth of embryonic and adult kidney tissue and uses for treating kidney damage. US-Patent, Application Number: 11807250, Filed on: 2007-05-25;, Inventors: Kai M. Schmidt-Ott, Jonathan Barasch, Jun Yang (via Columbia University)

Biomarkers for determination of temporal phase of acute kidney injury, PCT-Anmeldung, EP 2397559 A1, US 61/355,723, Filed on: 2010-06-17, Inventors: Kai M. Schmidt-Ott, Anne Wuebken (via MDC/Ascenion)

Selected Publications

Paragas N, Qiu A, Zhang Q, Samstein B, Deng SX, Schmidt-Ott KM, Viltard M, Yu W, Forster CS, Gong G, Liu Y, Kulkarni R, Mori K, Kalandadze A, Ratner AJ, Devarajan P, Landry DW, D'Agati V, Lin CS, Barasch J. The Ngal reporter mouse detects the response of the kidney to injury in real time. **Nature Medicine.** 2011;17(2):216-22.

Nickolas TL*, Schmidt-Ott KM*, Canetta P, Forster C, Singer E, Sise M, Elger A, Maarouf O, Sola-Del Valle DA, O'Rourke M, Sherman E, Lee P, Geara A, Imus P, Guddati A, Polland A, Rahman W, Elitok S, Malik N, Giglio J, El-Sayegh S, Devarajan P, Hebbar S, Saggi SJ, Hahn B, Kettritz R, Luft FC, Barasch J. Diagnostic and Prognostic Stratification in the Emergency Department Using Urinary Biomarkers of Nephron Damage - A Multicenter Prospective Cohort Study. **Journal of the American College of Cardiology.** 2012, 59:235-44. *** Co-First und Co-Corresponding authors.**

Singer E, Elger A, Elitok S, Kettritz R, Nickolas TL, Barasch J, Luft FC, Schmidt-Ott KM. Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. **Kidney International.** 2011;80(4):405-14.

Werth, M., Walentin, K., Aue, A., Schonheit, J., Wuebken, A., Pode-Shakked, N., Vilianovitch, L., Erdmann, B., Dekel, B., Bader, M., Barasch, J., Rosenbauser, F., Luft, F.C., and Schmidt-Ott, K.M. 2010. The transcription factor grainyhead-like 2 (Grhl2) regulates the molecular composition of the epithelial apical junctional complex. **Development.** 2010;137(22):3835-45..

Schmidt-Ott KM, Masckauchan TN, Chen X, Hirsh BJ, Sarkar A, Yang J, Paragas N, Wallace VA, Dufort D, Pavlidis P, Jagla B, Kitajewski J, Barasch J.?-catenin/TCF/Lef controls a differentiation-associated transcriptional program in renal epithelial progenitors. **Development.** 2007;134(17):3177-90.

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Photo: David Ausserhofer/MDC

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Zebrafish Cardiovascular Developmental Genetics

Vertebrate organs are derived from epithelial or endothelial sheets of cells that undergo complex morphogenetic transformations. The Seyfried lab studies the early zebrafish heart, a relatively simple organ compared with its mammalian counterpart, to better understand the signaling events that instruct the assembly of the early heart tube. Initially this organ consists of only the outer myocardial and inner endocardial cell layers. The group would like to understand: What are the signals that regulate the morphogenesis of myocardium and endocardium? To what extent do these two tissues communicate during cardiac jogging and looping morphogenesis? What determines the differentiation of endocardium into its different morphological derivatives such as cushion cells? In collaboration with clinical researchers, the group uses developmental genetics combined with cell biological and pharmacological approaches to develop animal models for human cardiovascular diseases. The group's long-term interest is to understand how the cellular mechanisms controlling zebrafish cardiogenesis shape the human heart and its associated blood vessels.

Control of cardiac laterality by combinatorial TGF-ß network signaling

Justus Veerkamp, Florian Priller, Zoltan Cseresnyes, Cécile Otten, Marc Renz

In an extension of previous studies on the cell biology of heart tube formation, we performed a comprehensive analysis of complex TGF-ß network signaling during early cardiac morphogenesis. Our highly-interdisciplinary approach included detailed expression analyses combined with functional studies, high-resolution live imaging, and mathematical modeling of cardiac jogging. We found that Nodal, via modulating the extracellular matrix within the left cardiac field, dampens the efficiency of BMP signaling on the left (Veerkamp et al., 2013). The mechanism by which Nodal modulates Bmp signaling activity involves the Nodal target Hyaluronan synthase 2 (Has2), which has an asymmetric and Spaw-dependent expression on the left side of the cardiac cone and is required for zebrafish cardiac laterality. Has2 is an enzyme involved in the production of hyaluronic acid, a major macromolecule of the extracellular matrix. Since high expression levels of *has2* on the left side of the cardiac cone correlate with dampened Bmp signaling activity, we tested whether clonal misexpression of *has2* within myocardial cells also reduces Bmp signaling activity. Indeed, clonal myocardial expression of *has2* resulted in strong dampening of Bmp activity. Comparative microarray expression analyses identified relevant cardiac effector genes of Bmp signaling that included cell adhesion, polarity and epithelial development factors, which were all in strong support of a role of Bmp signaling for cardiac progenitor cell adhesion. Our findings suggested that minor left-right differences in Bmp activity within the cardiac field may determine bi-phasic states: cardiac progenitor cells within the right cardiac cone being slightly more adhesive, and cells on the left side exhibiting a slightly more motile character. Thus, cardiac left-right asymmetry may be explained by Nodal modulating an anti-motogenic Bmp activity. In clonal misexpression studies, in which BMP activity was clonally reduced within the right cardiac field, we observed an inversion of cardiac laterality. Moreover, reducing BMP activity to below normal levels on the left side even enhanced cardiac jogging towards the left. One implica-

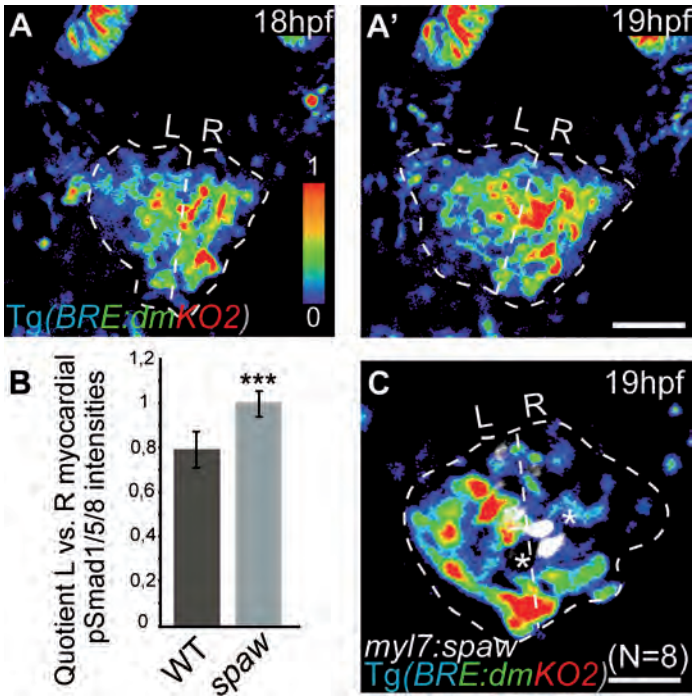


Figure 1: Effect of Nodal on L/R asymmetric BMP activity within the heart. *(A,A') Establishment of Bmp signaling asymmetry within the heart(outlined with white dotted line) between 16-21 hpf. The reporter line Tg(BRE-AAVmlp:dmKO2)^{mw40} drives expression of destabilized monomeric Kusabira-Orange 2 (dmKO2) which indicates Smad-1/5/8-mediated activity. (B) The L/R asymmetry of nuclear pSmad-1/5/8 intensities in WT, as indicated by the quotient of L- versus R-sided pSmad-1/5/8 intensities, is abolished in spaw morphant embryos at 19 hpf (***, p<0.0005). (C) Reversal of Bmp activity and cardiac laterality upon misexpression of spaw within the right cardiac field (asterisks, GFP false-colored in white). Signaling intensities are indicated by color range*

tion of these clonal studies was that changes in individual cell motility rates impact the tissue displacement of larger coherent groups of cells. Indeed, in silico modeling confirmed that left-right differences in cell motility are sufficient to induce robust directional migration of cardiac tissue. Based on these experiments, we propose that aspects of cardiac laterality may be explained by a random cell motility gradient mechanism.

Establishment of a neuroepithelial barrier by Claudin5a is essential for zebrafish brain ventricular lumen expansion

Jingjing Zhang, Cécile Otten (in collaboration with Ingolf Blasig, Leibniz Institute for Molecular Pharmacology, Berlin)

Formation and physiology of the zebrafish neuroepithelial-ventricular barrier is tightly coupled with neuroepithelial maturation. In all vertebrates, the embryonic neuroepithelium is initially organized as a columnar epithelial sheet which undergoes neurulation to form the neural tube. Previous studies in zebrafish had already demonstrated that the process of brain morphogenesis, which involves the expansion of brain ventricles, requires coherence among neuroepithelial cells via apical junctional complexes. Furthermore, zebrafish mutants with neuroepithelial tissue polarity defects or with Cadherin-based cell-cell adhesion defects fail to expand brain ventricular lumens. In functional *knock-down* studies, we showed that zebrafish Claudin-5a regulates the permeability properties of neuroepithelial cell tight junctions as assessed by injection of the small electron-dense tracer molecule lanthanum nitrate into brain ventricles between 17-20 hours post fertilization. This correlated with a failure of brain ventricles to expand in embryos deficient for Claudin-5a. Strikingly, this effect was not caused by a general loss of neuroepithelial tissue polarity since the apical localization of the tight junctions associated marker protein ZO-1 and the normal distribution of the Atp1 α1 ion pump were not affected. These analyses demonstrated that the early neuroepithelial layer lining the brain ventricles represents an embryonic cerebral-ventricular barrier and that Claudin-5a determines the permeability of hindbrain neuroepithelial cell tight junctions for small molecules (molecular mass of lanthanum nitrate: 0.5 kDa). However, since brain ventricular lumen expansion was not entirely blocked by loss of Claudin-5a, other Claudins may potentially contribute to the tightening of the neuroepithelial barrier as well.

Selected Publications

Veerkamp J, Rudolph F, Cseresnyes Z, Priller F, Otten C, Renz M, Schaefer L, Abdelilah-Seyfried S. (2013). Unilateral dampening of Bmp activity by nodal generates cardiac left-right asymmetry. Dev Cell. 24:660-7.

Wieffer M, Cibrián Uhalte E, Posor Y, Otten C, Branz K, Schütz I, Mössinger J, Schu P, Abdelilah-Seyfried S, Krauß M, Haucke V. (2013). PI4K2β/AP-1-based TGN-endosomal sorting regulates Wnt signaling. Curr Biol. 23:2185-90.

Tay HG, Schulze SK, Compagnon J, Foley FC, Heisenberg CP, Yost HJ, Abdelilah-Seyfried S, Amack JD. (2013). Lethal giant larvae 2 regulates development of the ciliated organ Kupffer's vesicle. Development. 140:1550-9.

de Pater E, Ciampricotti M, Priller F, Veerkamp J, Strate I, Smith K, Lagendijk AK, Schilling TF, Herzog W, Abdelilah-Seyfried S, Hammerschmidt M, Bakkers J. (2012). Bmp signaling exerts opposite effects on cardiac differentiation. Circ Res. 110:578-87.

Zhang, J, Piontek, J, Wolburg, H, Piehl, C, Liss, M, Otten, C, Christ, A, Willnow, TE, Blasig, IE, Abdelilah-Seyfried, S.(2010). Establishment of a neuroepithelial barrier by Claudin5a is essential for zebrafish brain ventricular lumen expansion. Proc. Natl. Acad. Sci USA, 107:1425-30.

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Molecular and Cellular Basis of Embryonic Development

Understanding how distinct cell types arise from multipotent progenitor cells is a major quest in stem cell biology. The endoderm germ layer gives rise to a number of vital organs, including the lungs, liver, pancreas and intestine. This remarkable diversity derives from a homogenous population of multipotent cells. Our goal is to elucidate how liver and pancreas arise from common endoderm progenitors in the embryo and acquire specialized shape to form functional organs. This fascinating question in developmental biology has direct clinical relevance. In the long run, our investigations will have direct implications in developing novel strategies to generate pancreas progenitors and β-cells either from programming of stem cells or from re-programming of adult hepatic cells, closing the gap between studies of basic processes in model systems and clinical research.

Comprehensive analysis of the hepatic and pancreatic lineage divergence

The pancreas consists of two organs in one: the exocrine compartment, which produces digestive enzymes, and the endocrine compartment, which houses the insulin-secreting β-cells and is critical for blood glucose homeostasis. Loss or dysfunction of β-cells results in diabetes, a still incurable metabolic disorder. Currently, much effort is targeted toward generating renewable sources of β-cells for cell replacement therapy of diabetes, and these efforts rely on the knowledge of pancreas development. The pancreas is closely related to the liver in terms of embryonic origin and gene regulatory network. Our research aims at investigating the origin of the pancreas and liver divergence at the cellular and molecular level. We used RNASeq to define the molecular identity of liver and pancreas progenitor cells isolated by FACS from the mouse embryo at the time of the lineage divergence (E8.5-E10.5). By integrating the temporal and spatial gene expression profiles, we defined mutually exclusive signaling signatures in hepatic and pancreatic progenitors. Deciphering the molecular signature of particular progenitor cell types holds the promise of isolating such cells for therapeutic purposes and, possibly, of instructing stem cells to activate appropriate differentiation programs. For instance, our results have provided us with guidelines not only on how to generate hepatocytes and pancreatic β-cells from human ES and iPS cells but also to formulate strategies to promote liver-to-pancreas lineage reprogramming. Notably, we have uncovered a unique non-canonical Wnt signaling signature in the emergence of pancreas versus liver from endoderm progenitors, which appears conserved across phylogenetically distant species. In addition to the Wnt-signature, we identified TALE homeoproteins, being differentially expressed between pancreatic and hepatic progenitor cells; and epigenetic modulators, as potential regulators of cell identity.

Lineage reprogramming approaches to generate functional pancreatic cells

Our previous studies provided novel insights into cellular plasticity and the basis for formulating reprogramming strategies between liver and pancreatic cells. Targeted functional studies of developmental regulators of the liver and pancreas fate decision and their lineage reprogramming activity are ongoing in my group. We focus on both extrinsic and intrinsic regulators of this cell fate decision.

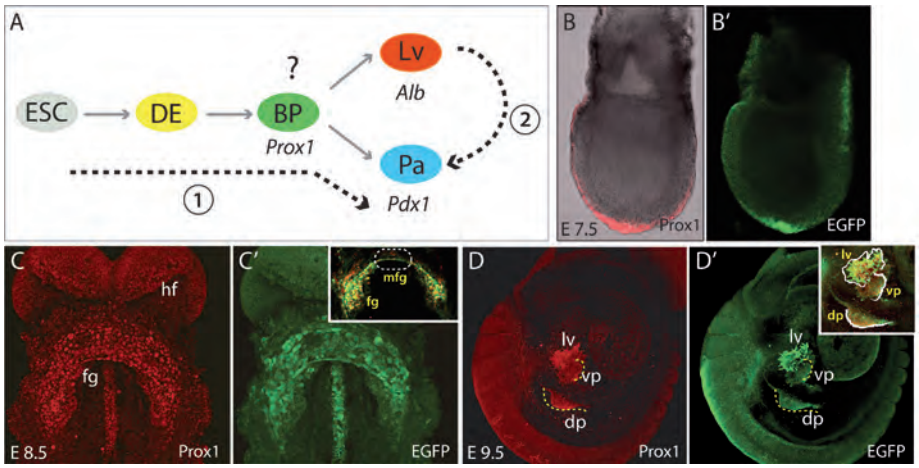


Figure 1. *(A) Schematic of hepatic and pancreatic cell lineage specification (1) and fate conversion (2). (B-D) Characterization of Tg(Prox1-EGFP) mouse embryos. Prox1 is the earliest specific marker in common between hepatic and pancreatic endoderm from gastrulation onwards. Genetically labeled Prox1-EGFP+ hepatic and pancreatic progenitors were purified by FACS at distinct developmental stages (E8.5-E10.5 interval) and analyzed by RNA-Seq. In insets, endoderm regions analyzed by RNA-Seq. Abbreviations: BP, bipotent progenitor; DE, definitive endoderm; fg, foregut; mfg, mid-foregut; hf, head fold; lv, liver; pa, pancreas; dp, dorsal pancreas; vp, ventral pancreas. (adapted from Rodriguez-Seguel et al. 2013).*

How and when **extrinsic factors**, such as BMPs, control the emergence of liver *versus* pancreas from the same embryonic region? To address this question, we have developed genetic approaches to perturb the signaling *in vivo* in a controlled temporal and spatial fashion. These experiments will elucidate: if BMP exerts a role *in vivo* in hepatic *versus* pancreatic fate decision in mammals; and different potential functions of BMP in the endoderm according to the embryonic stages. Moreover, we have identified the TALE homeoprotein TGIF2 as an **intrinsic regulator** of the pancreas *versus* liver fate decision in the endoderm and whose expression is sufficient to convert differentiated liver cells to a pancreatic progenitor state. This study uncovers the reprogramming activity of TGIF2 and opens to new investigation into the mechanistic aspects of the liver-to-pancreas lineage conversion.

Control of morphogenesis and tissue-architecture in the developing pancreas

During organogenesis, tissue-architecture defines particular local microenvironments, which in turn impact progenitor specification and subsequent differentiation. For example, the distal tips of the branching pancreas epithelium contain fast proliferating progenitors and, possibly, define a special supporting "niche". How branching morphogenesis occurs and is coordinated with proliferation and differentiation in the pancreas is largely unknown. We have developed mouse models and time-lapse imaging of *ex vivo* embryonic pancreas cultures to study morphogenetic, proliferation and differentiation events as they occur in the developing embryo. Using these approaches, we showed that Rho and its negative regulator, the RhoGAP protein Stard13, control branching morphogenesis of the pancreatic epithelium. Conditional ablation of *Stard13* expression in the mouse pancreatic epithelium disrupts morphogenesis and tip domain organization, resulting in hampered proliferation of tip progenitor cells and, subsequent, organ hypoplasia. The Stard13 mutant phenotype analysis has raised many interesting questions about cellular mechanisms controlling the formation of the tips and progenitor allocation. Novel lines of research in my lab are aimed at investigating **1)** the epithelial dynamics of branching morphogenesis and **2)** temporal and spatial allocation of pancreatic tip progenitor cells. In the long run, we expect that a full comprehension of the 3D organization of the pancreatic tissue will have practical implications in any attempt of cell-based therapy of diabetes based on *ex vivo* expansion of pancreatic progenitors and generation of insulin producing β-cells either from stem cells or lineage reprogramming.

Selected Publications

Rodríguez-Seguel E, Mah N, Naumann N, Pongrac IM, Cerdá-Esteban N, Fontaine J-F, Wang Y, Chen W, Andrade-Navarro MA, Spagnoli FM. "Mutually exclusive signaling signatures define the hepatic and pancreatic progenitor cell lineages divergence" Genes & Development 2013; Vol. 27: 1932–1946.

Cerdá-Esteban N, Spagnoli FM. "A Glimpse Into Hox and TALE Regulation of Cell Differentiation and Reprogramming" Developmental Dynamics 2014; Vol. 243: 76-87.

Petzold K, Naumann H, Spagnoli FM. "Rho signaling restriction by the RhoGAP Stard13 integrates growth and morphogenesis in the pancreas" Development 2013; Vol. 140: 126-135.

Gong M, Simaite D, Kühnen P, Heldmann M, Spagnoli FM, Blankenstein O, Hübner N, Hussain K, Raile K. "Two novel GATA6 mutations cause childhood-onset diabetes mellitus, pancreas malformation and congenital heart disease" Horm. Res. Paediatr. 2013; Vol. 79: 250-6.

Petzold K, Spagnoli FM. "A System for Ex vivo Culturing of Embryonic Pancreas" J. Vis. Exp. 2012; Vol. 66: e3979.

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Photo: David Ausserhofer/MDC

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Molecular Cardiovascular Research

We study orphan endocytic receptors to uncover their roles in normal physiological processes and in diseases. Recently, we demonstrated that LRP2, a member of the LDL receptor gene family, acts as a receptor for sonic hedgehog, and is required for signaling of this morphogen in the mammalian forebrain. Lack of LRP2 activity, in mouse models and in patients, results in forebrain malformations and in holoprosencephaly. We also characterized SORLA and sortilin, members of a novel class of neuronal endocytic receptors. We showed that both receptors control distinct steps in the catabolism of the Aβ peptide, the causative agent in Alzheimer's disease (AD). We found that SORLA acts as an intracellular sorting receptor for Aβ that directs nascent peptides to lysosomal degradation, while sortilin clears Aβ from the brain interstitial fluid (ISF) when bound to apolipoprotein E. Both receptors effectively reduce the amount of neurotoxic Aβ in the brain and constitute promising drug targets for the treatment of AD.

Introduction

Receptor-mediated endocytosis is the main mechanism for selective transport of macromolecules into cells. Significant progress has been made in elucidating the various steps of endocytosis at the cellular level. However, the physiological relevance of many endocytic pathways for organ (dys) function remains elusive.

The main class of endocytic receptors is the LDL receptor gene family (Fig. 1). The prototype of this gene family is the LDL receptor that mediates cellular uptake of cholesterol-rich lipoproteins. Since other family members also bind lipoproteins, roles for these receptors in lipid metabolism had been anticipated. Surprisingly, studies uncovered many more functions performed by these receptors, changing our perception of lipoprotein receptors from mere cargo transporters to key regulators of numerous physiological processes. In particular, the significance of these receptors for signal transduction in embryonic development is noteworthy. Yet, studies so far provide just a glimpse at their contributions to developmental processes, with many details still to be uncovered.

Whereas studies on the LDL receptor gene family yielded exciting insights into the significance of endocytosis for cell signaling, identification of another group of orphan receptors called VPS10P domain receptors or Sortilins directed our attention to intracellular protein transport. The founding member of this gene family is SORLA, a receptor that shares structural elements of the LDL receptor gene family (Fig. 1). In addition, it includes a domain identified in VPS10P, a sorting protein in yeast. Subsequent identification of four other mammalian proteins that share the VPS10P domain uncovered the existence of a new class of receptors that act in endocytosis and/or intracellular protein transport. Their many functions remain yet to be identified.

LRP2 is an auxiliary SHH receptor required for forebrain development

Annabel Christ, Anna Christa

LRP2 is a receptor expressed in the neural tube, the embryonic tissue that gives rise to the central nervous system. Early on, we recognized the importance of LRP2 for

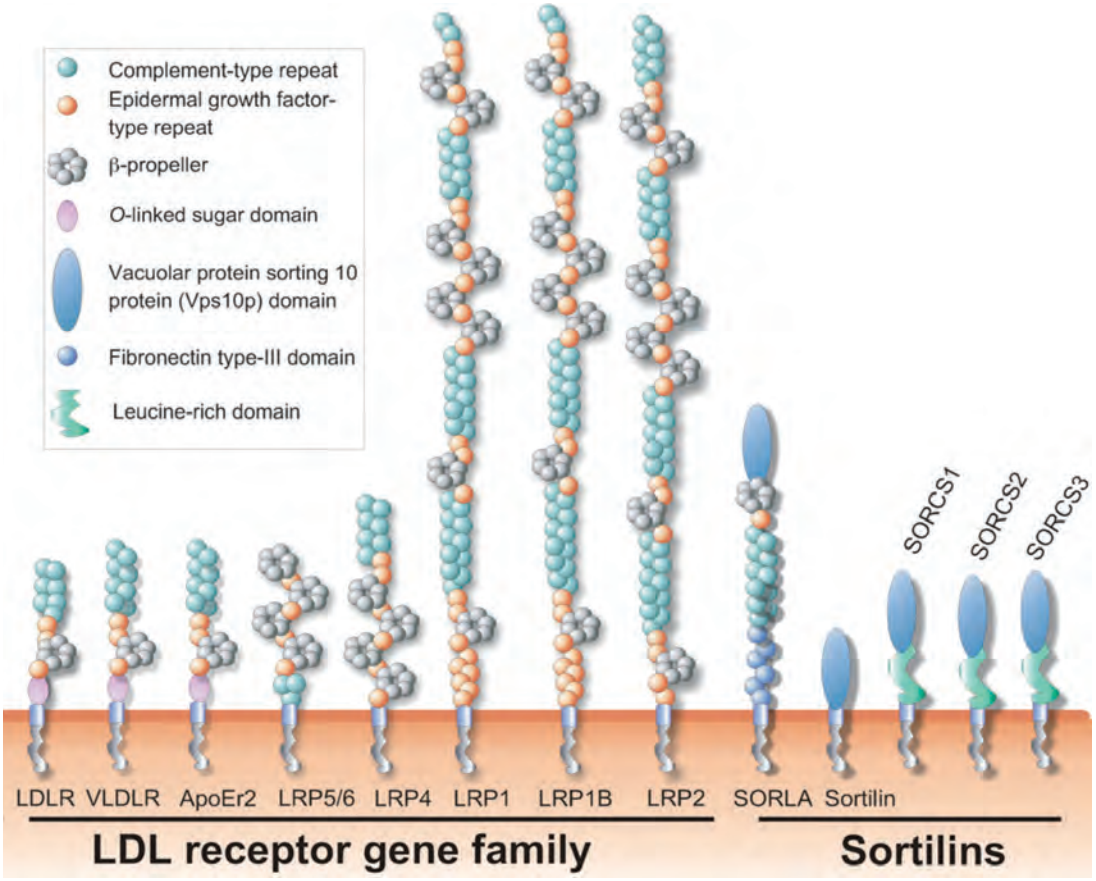


Fig. 1:
The structural organization of members of the LDL receptor and VPS10 domain receptor (Sortilins) gene families is shown.

brain development as mice genetically deficient for the receptor suffer from holoprosencephaly, a defect in the separation of the forebrain hemispheres and the most common forebrain anomaly in humans. The significance of this receptor for humans was confirmed by others, demonstrating LRP2 defects as the cause of Donnai-Barrow syndrome, a monogenic disorder characterized by forebrain anomalies.

Recently, we succeeded in elucidating the mechanism of LRP2 action in brain development (Christ et al., Developmental Cell 2012; Willnow et al., Development 2012). Specifically, Annabel Christ traced the primary defect in *Lrp2*$^{-/-}$ embryos to a failure to establish signaling of the morphogen sonic hedgehog (SHH) in the rostral diencephalon ventral midline (RDVM), a major forebrain organizer. At neurulation, SHH originating from the prechordal plate moves to the overlying RDVM to induce its own expression and to establish its signaling domain (Fig. 2). Annabel demonstrated that in *Lrp2* mutants, SHH fails to localize to the RDVM, suggesting a defect in morphogen sequestration. Using cell lines, whole embryo cultures, and neural explants, Annabel and Anna further refined this pathway, ultimately demonstrating that LRP2 acts as auxiliary SHH receptor that sequesters this morphogen in its target field and enables signaling of SHH through its cognate receptor patched 1, a step essential to induce forebrain patterning (Fig. 2)
Our data identified a novel concept in morphogen signaling and revealed the molecular mechanism underlying forebrain anomalies in patients with Donnai-Barrow syndrome. Our ongoing studies focus on exploring the relevance of this novel signaling pathway for other clinically relevant aspects of SHH signaling as in retinal degeneration or cancer.

SORLA controls production of Aβ

Vanessa Schmidt, Tilman Burgert, Safak Caglayan

Aβ is a neurotoxic peptide produced by proteolytic processing of the amyloid precursor protein (APP). Excessive production of Aβ is held responsible for the synaptic dysfunction and memory impairment observed in patients with AD. Accordingly, mechanisms that reduce Aβ levels represent promising targets for prevention of this devastating disease.

Previously, we documented the ability of SORLA to act as an intracellular sorting receptor for APP that prevents trafficking of this precursor into intracellular compartments where processing into Aβ occurs. These findings suggested that the low SORLA levels seen in some individuals

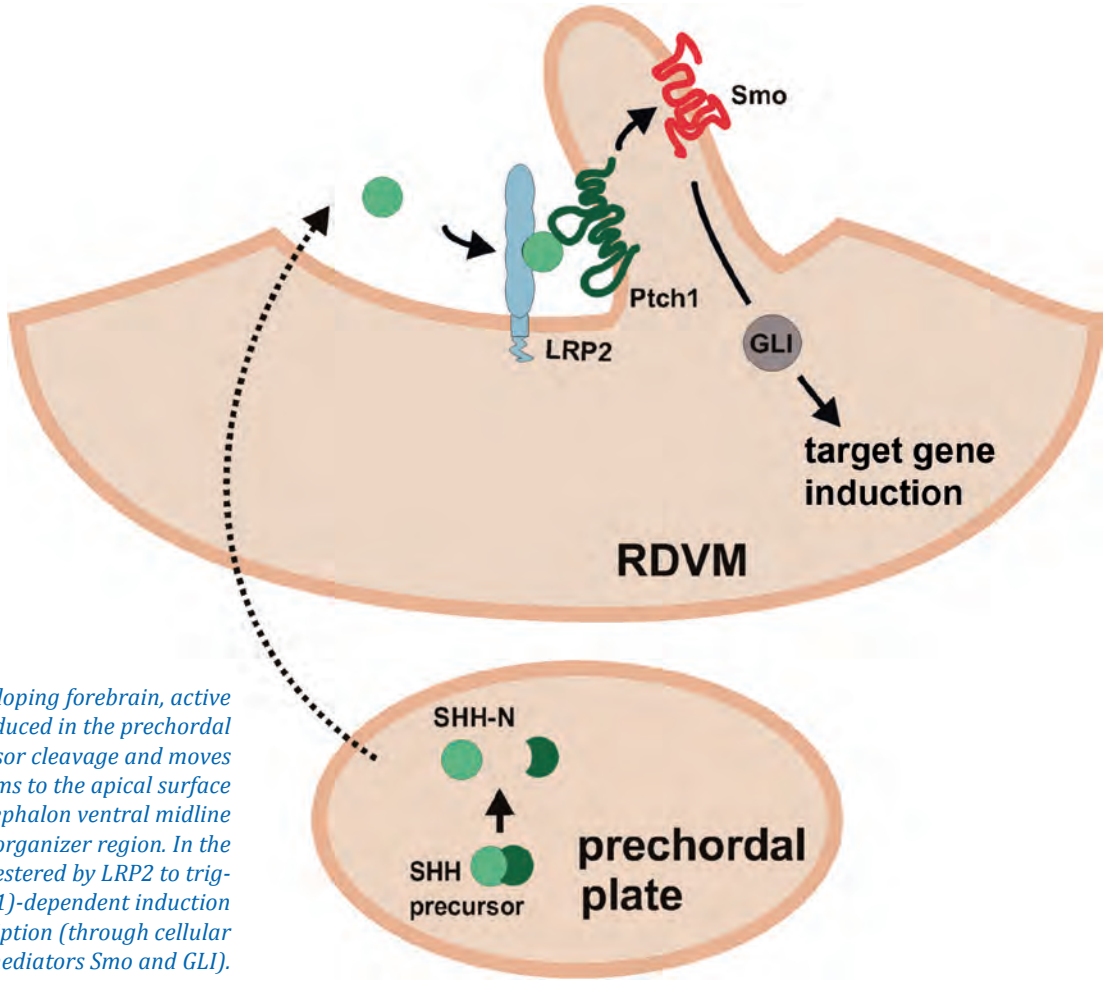


Fig. 2: *In the developing forebrain, active SHH (SHH-N) is produced in the prechordal plate by SHH precursor cleavage and moves by unknown mechanisms to the apical surface of the rostral diencephalon ventral midline (RDVM), a forebrain organizer region. In the RDVM, SHH-N is sequestered by LRP2 to trigger patched 1 (Ptch1)-dependent induction of target gene transcription (through cellular mediators Smo and GLI).*

with AD may be the underlying cause of enhanced Aβ production. To confirm the inverse correlation of SORLA levels with Aβ production rates, Vanessa Schmidt and her colleagues combined quantitative biochemical studies with mathematical modeling to establish a kinetic model of amyloidogenic processing and to evaluate the influence by SORLA on this process. Their studies demonstrated that secretases (the proteases cleaving APP) represent allosteric enzymes that require cooperativity by APP oligomerization for efficient processing. Cooperativity enables swift adaptive changes in secretase activity with even small alterations in APP concentration. They also showed that SORLA prevents APP oligomerization eliminating the preferred form of the substrate and causing secretases to switch to a less efficient non-allosteric mode of action (Schmidt et al., EMBO J 2012).

Given the importance of SORLA-dependent trafficking of APP for Aβ production, Tilman Burgert set out to elucidate the molecular mechanisms controlling SORLA sorting in neurons. Using mouse models that express trafficking mutants of SORLA, he and his collaborators identified that SORLA interacts with the cytosolic adaptor PACS1 that shuttles SORLA/APP complexes from endosomes to the Golgi, reducing the extent of APP processing in endosomal compartments. Mutating the binding site for PACS1 in the cytoplasmic domain of SORLA resulted in aberrant retention of this receptor (and its target APP) in endosomes and, consequently, in enhanced Aβ production (Burgert et al., MCB 2013).

To provide proof that overexpression of SORLA reduces Aβ levels *in vivo,* Safak Caglayan generated mice that overexpress this receptor. In line with our hypothesis, increasing levels of SORLA in the brain profoundly reduced Aβ burden in these animals. Exploring the underlying mechanism, Safak and his colleagues made the surprising observation that SORLA not only acts as sorting receptor for APP, but also moves newly synthesized Aβ molecules to lysosomes for degradation, reducing the amount of neurotoxic peptides released by neurons (Caglayan et al., Science Trans Med, in press). Safak also documented that the ability of SORLA to sort Aβ is lost in a re-

ceptor mutant expressed in a familial form of AD. His studies substantiated SORLA as a potential drug target in AD and the loss of receptor function as a possible cause of early-onset AD.

Sortilin mediates clearance of Aβ in the brain

Anne-Sophie Carlo

In the brain, astrocytes secrete lipoproteins containing apolipoprotein E (apoE) that are cleared from the brain ISF by apoE receptors expressed in neurons. These uptake pathways provide neurons with essential cholesterol (transported in lipoprotein particles) to sustain membrane integrity and synaptogenesis. Intriguingly, APOE is also the most important genetic risk factor for sporadic AD. Among other functions, APOE is proposed to sequester Aβ molecules in the ISF, delivering them to cellular catabolism via APOE receptors. The receptors involved in this process remain controversial. Because we had previously identified sortilin as an apolipoprotein receptor in the liver, Anne-Sophie Carlo tested whether this protein may also be an apoE receptor in neurons. In line with her hypothesis, she showed that sortilin binds APOE with high affinity. Lack of sortilin expression in mice resulted in the accumulation of APOE and of Aβ in the brain, as well as in an aggravated amyloidogenic burden. Sortilin-deficient animals displayed anomalies in brain lipid metabolism seen in APOE-deficient mice, indicating functional deficiency in cellular APOE uptake pathways (Carlo et al., J Neurosci 2013; Nykjaer and Willnow, Trends Neurosci 2012). Taken together, these findings identified sortilin as a neuronal pathway for APOE-containing lipoproteins *in vivo* and uncovered the relevance of this receptor for brain lipid homeostasis and AD.

Outlook

Dysregulation of protein transport is emerging as a molecular mechanism of major importance in many disease processes. Among other functions, faulty transport impacts cellular signal reception (as for SHH), results in abnormal protein homeostasis (as for APP processing), or alters extracellular levels of metabolites (as for Aβ). Our data have shown that receptors of the LDL and VPS10P domain receptor gene families play key roles in these disease processes. Our future work aims at refining the molecular details how these receptors affect trafficking and functional expression of target molecules and how receptor dysfunctions contribute to the most devastating defects in brain development and function occurring in humans.

Selected Publications

Caglayan, S., Takagi-Niidome, S., Liao, F., Carlo, A.-S., Schmidt, V., Burgert, T., Kitago, Y., Füchtbauer, E.-M., Füchtbauer, A., Holtzman, D.M., Takagi, J., and T.E. Willnow. Novel role for SORLA/SORL1 as lysosomal sorting receptor for amyloid-beta peptide is impaired by familial Alzheimer disease mutation. Science Trans Med (in press)

Carlo, A.-S., Gustafsen, C., Mastrobuoni, G., Nielsen, M.S., Burgert, T., Hartl, D., Rohe, M., Nykjaer, A., Herz, J., Heeren, J., Kempa, S., Petersen, C.M., and T.E. Willnow. (2013) The Pro-Neurotrophin Receptor Sortilin Is a Major Neuronal Apolipoprotein E Receptor for Catabolism of Amyloid-β Peptide in the Brain. J Neurosci 33: 358-70.

Nykjaer A., and T.E. Willnow. (2012). Sortilin, a receptor to regulate neuronal viability and function. Trends Neurosci 35: 261-70.

Schmidt V., Baum, K., Lao, A., Rateitschak, K., Schmitz, Y., Teichmann, A., Wiesner, B., Petersen, C.M., Nykjaer, A., Wolf, J., Wolkenhauer, O., and T.E. Willnow. (2012). Quantitative modeling of amyloidogenic processing and its influence by SORLA in Alzheimer's disease. EMBO J 31: 187-200.

Christ, A., Christa, A., Kur, E., Lioubinski, O., Bachmann, S., Willnow, T.E.*,and Hammes, A. (2012). LRP2 is an auxiliary SHH receptor required to condition the forebrain ventral midline for inductive signals. Dev Cell 22: 268-278. *corresponding author

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Photo: David Ausserhofer/MDC

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Video:
What can we learn from counting molecules?

Mathematical Modelling of Cellular Processes

Complex diseases are often characterised by an accumulation of multiple perturbations in rather large and complex cellular networks. The consequences of these perturbations, such as mutations or over-expression of proteins, can hardly be analysed by pure reasoning. Here, mathematical modelling contributes to a deeper understanding of the regulatory systems and provides thus a better basis for the interpretation of high-throughput data and identification of effective drug targets.
Our group develops and analyses mathematical models of signalling pathways and gene-regulatory networks in normal and disease states. For our investigations we use tools such as simulations, bifurcation analyses and sensitivity analyses. These give insights into the dynamical properties of the systems and help to identify most sensitive processes and critical regulations. Another important aspect is the investigation of cell specific differences in signalling and gene-regulatory networks since these are critically involved in the prediction of the efficiency and possible side-effects of drugs.

Modelling mammalian signal transduction pathways: IKK/ NF-κB signalling and Wnt/ β-catenin signalling

In a collaborative approach with the group of Claus Scheidereit at the MDC we aim for a systems level understanding of the IKK/ NF-κB signalling pathway. This pathway consists of a canonical and a non-canonical branch. Both have a distinct timing and distinct biological functions but are interconnected. On the one hand substrates and inducers of the non-canonical branch are produced in the canonical branch, on the other hand a control of the canonical part by the non-canonical branch was reported. We are interested in the regulation of the long-time behaviour of the overall pathway and its malfunction in diseases, e.g. Hodgkin-lymphoma. In particular, we want to dissect the contribution of canonical and non-canonical modules under these conditions. To that end we are investigating the kinetic properties, feedback regulations and interacting modules of both signalling branches.

The Wnt/ β-catenin pathway is another important signal transduction pathway. Its deregulation is associated with various types of cancer. We use mathematical modelling of the canonical Wnt/ β-catenin pathway to analyse the effect of transcriptional feedbacks (e.g. via Axin, β-TrCP/ HOS) and the cross-talk of Wnt/ β-catenin signalling to other pathways.

Robustness of cellular rhythms

Many examples for rhythmic phenomena can be found on the cellular level, e.g. circadian rhythms, the cell cycle, calcium or metabolic oscillations. These rhythms are involved in various functions and react specifically to environmental changes. Our project addresses the question of what determines the balance between sensitivity and robustness of a cellular rhythm. To this end we compare the dynamical properties of various well-established mathematical models for different rhythms. We find models for calcium oscillations to be very sensitive, those for circadian rhythms very robust. These findings correspond well to the biological functions since for calcium oscillations a period-encoded signal transduction was shown, whereas circadian oscillations generate an internal time information. In a subsequent analysis, the impact of local kinetic parameters versus that of the system topology on the robustness is studied.

Metabolic synchronisation by travelling waves in cell layers

The coordination of cellular dynamics is a prerequisite of the functionality of tissues and organs. Generally, this coordination may occur by signal transduction, neuronal control, or exchange of messenger molecules. The extent to which metabolic processes are involved is less understood. Previously, a communicaton between cells via the exchange of metabolites, called dynamic quorum sensing, was shown in stirred cell suspensions. However, it was an open question whether the coupling of cells without stirring would be sufficient for the coordination of cellular behaviour. We studied this question by an experimental-theoretical approach using a simple experimental system, that is, a layer of resting yeast cells. We could for the first time show that a local addition of substrate led to the formation of intercellular glycolytic waves in these cell layers. The theoretical analysis of this phenomenon showed that the radial wave velocity arises from the substrate gradient. Overall, the results demonstrate that metabolic processes introduce an additional level of local intercellular coordination.

Selected Publications

Benary U.; Kofahl B.; Hecht A.; Wolf J. (2013), Modeling Wnt/beta-catenin target gene expression in APC and Wnt gradients under wild type and mutant conditions, Frontiers in Physiology 4: 21.

Schmidt V.; Baum K.; Lao A.; Rateitschak K.; Schmitz Y.; Teichmann A.; Wiesner B.; Petersen C.M.; Nykjaer A.; Wolf J.; Wolkenhauer O.; Willnow T.E. (2012), Quantitative modelling of amyloidogenic processing and its influence by SORLA in Alzheimer's disease, EMBO Journal 31 (1): 187-200.

Schwanhaeusser B.; Busse D.; Li N.; Dittmar G.; Schuchhardt J.; Wolf* J.; Chen* W.; Selbach* M. (2011), Global quantification of mammalian gene expression control , Nature 473 (7347): 337-342 (* corresponding authors).

Schuetze J.; Mair T.; Hauser M.J.; Falcke M.; Wolf J. (2011), Metabolic synchronization by traveling waves in yeast cell layers, Biophysical Journal 100 (4): 809-813.

Kofahl B.; Wolf J. (2010), Mathematical modelling of Wnt/beta-catenin signalling. Biochemical Society Transactions 38 (5): 1281-1285.

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Photo: David Ausserhofer/MDC

Michael Bader

Cardiovascular Hormones

The group focuses on the molecular biology and function of hormone systems involved in cardiovascular regulation. The physiological and metabolic functions of these systems are evaluated by the production and analysis of transgenic and gene-targeted animal models. Among the hormones studied, serotonin is of special interest, since it is not only involved in vascular homeostasis and other peripheral functions, but also serves as potent and multifunctional neurotransmitter in the brain. In addition, the group is interested in embryology and stem cell research, in particular applying these fields to the rat.

Renin-angiotensin system

Natalia Alenina, Diogo Cavalcanti, Carlos Fraga, Irina Lapidus, Valeria Nunes, Gabin Sihn, Rafael Morais, Daisy Motta, Aline Oliveira, Luiza Rabelo, Martijn Rotteveel, Anthony Rousselle, Laura Souza, Mihail Todiras

The renin-angiotensin system (RAS) is of central importance in blood pressure regulation and in the initiation of target organ damage. In particular, local angiotensin-II generating systems in tissues such as brain, heart, vessels, and kidney are involved in these processes. Therefore, transgenic rats with local up- or downregulation of RAS components in these organs, e.g. by the local expression of antisense-RNA or of a peptide-liberating protein, were produced and analyzed to clarify the local functions of angiotensin II. Other genetically altered mouse and rat models for non-classical RAS components such as ACE2, the (pro)renin receptor, angiotensin(1-7) and its receptor Mas, have elucidated the physiological function of these molecules. Moreover, a novel peptide of the RAS, alamandine, with its receptor, MrgD, was discovered. Together with transgenic rats overexpressing ACE2 or angiotensin(1-7), ACE2- and Mas-knockout mice characterized the ACE2/angiotensin(1-7)/Mas system as a cardioprotective axis that counteracts the classical RAS effects in particular improving endothelial function. Moreover, these animals showed that angiotensin(1-7) and Mas are important for insulin sensitivity and the pathogenesis of metabolic syndrome. In a newly established transgenic rat model with inducible diabetes mellitus type 2 a novel oral formulation of angiotensin(1-7) was shown to exert potent antidiabetic actions revealing the therapeutic potential of the protective RAS axis.

Kallikrein-kinin system and chemokines

Carlos Barros, Mike Becker, Johan Duchene, Lorena Gomez, Paola Guimaraes, Anne Järve, Silke Mühlstedt, Katrin Nitz, Fatimunnisa Qadri

The kallikrein-kinin system (KKS) is an important hormone system for cardiovascular regulation also mostly counteracting the effects of the RAS. As models for the functional analysis of the KKS in intact animals, transgenic rats were generated expressing different components of the system, such as tissue kallikrein, the kinin B1 or the B2 receptor either ubiquitously or specifically in cardiovascular organs. These animals supported the protective role of the KKS in kidney and heart against ischemic, diabetic, and hypertrophic injury. Knockout mice for the kinin B1 receptor were generated and revealed important functions of this protein in pain perception and inflammation. Moreover, the B1 receptor turned out to be

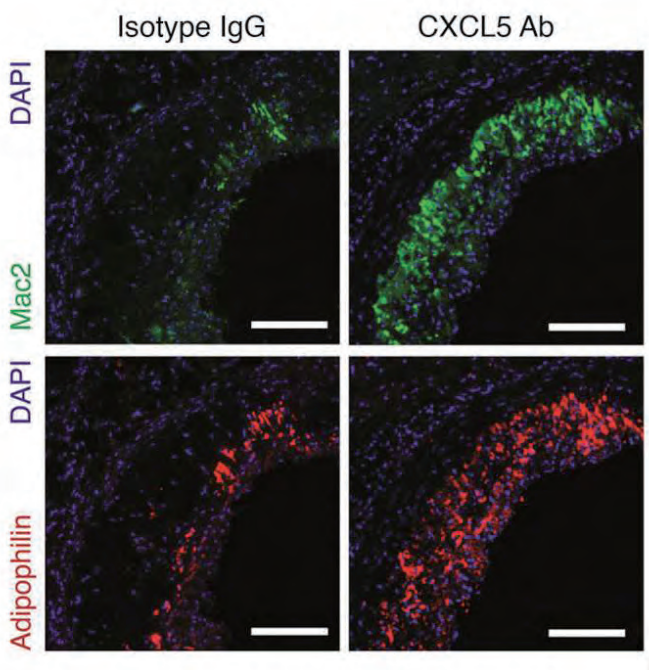


Figure 1: CXCL5 blockade increases macrophage foam cell formation in atherosclerosis
Blockade of CXCL5 is associated with macrophage foam cell accumulation in atherosclerotic plaques in Apolipoprotein E deficient mice fed a Western diet and treated with either IgG isotype control (IgG) or anti-CXCL5 Ab (CXCL5 Ab) for 12 weeks. Staining for DAPI (nuclei), Mac2 (macrophages, upper panels), and adipophilin (fat containing vesicles, lower panels). Scale bars: 100 µm.

involved in arteriogenesis, sepsis, stroke, multiple sclerosis and high-fat diet induced obesity. Mice lacking both kinin receptors and thereby being devoid of a functional KKS were also generated and shown to be completely normal at baseline suggesting that the KKS is irrelevant for development and the basic regulation of the cardiovascular system but is involved in the pathogenesis of multiple diseases and, thus, a relevant drug target.

Moreover, downstream mediators of kinins such as the chemokine CXCL5 and its receptor CXCR1 as well as other chemokines, such as CXCL12, are functionally analyzed in newly developed knockout mouse models. Mice with a local deletion of CXCL12 in cardiomyocytes presented an unexpected deteriorating role of this chemokine for the heart function after cardiac damage. CXCL5 was characterized as major determinant of macrophage foam cell formation in atherosclerosis.

Serotonin system

Natalia Alenina, Annika Balke, Saleh Bashammakh, Daniel Beis, Michael Flinders, Yasmine Graf, Maik Grohmann, Cornelia Hainer, Friederike Klempin, Susann Matthes, Valentina Mosienko, Ashish Ranjan, Magdalena Zaniewska

Serotonin is a monoamine which functions as an important neurotransmitter in the central nervous system and as a major peripheral mediator produced by enterochromaffin cells of the gut and transported and released by platelets in the circulation. We discovered that vertebrates have two genes coding for tryptophan hydroxylase, the rate-limiting enzyme in serotonin synthesis, TPH1 and TPH2. Mice deficient in TPH1, the isoform responsible for the synthesis of serotonin in the gut, showed that peripheral serotonin is involved in thrombosis, pulmonary hypertension, remodelling of mammary glands, tumor angiogenesis, liver regeneration, and hepatitis, but not in bone metabolism as previously suggested. Mice deficient in TPH2, the isoform responsible for the synthesis of serotonin in the brain, were surprisingly viable and fertile, despite a near complete lack of serotonin in the brain, and showed growth retardation and altered autonomic control leading to impairment of sleep, respiration, cardiovascular and metabolic parameters. Our recent data revealed that exercise-induced neural cell proliferation in the dentate gyrus (adult neurogenesis) is abolished in animals with life-long depletion in brain serotonin. In addition, TPH2-deletion is correlated with increased aggression, maternal neglect, loss of aversive and anxiety-like behavior and depression-like phenotypes in mice. Since further behavior analysis of this model was hampered by poor ability of the mouse for complex social interactions and learning capacity, we now perform these studies in TPH2-deficient rats generated via zinc-finger-nuclease (ZFN) based gene-deletion. Since brain serotonin is a major determinant in the pathogenesis of psychiatric disorders, such as depression, we develop modulators of TPH activity as novel therapeutic drugs together with the high-throughput screening platform of the Research Institute for Molecular Pharmacology (FMP) and the Helmholtz Protein Sample Production Facility (PSPF).

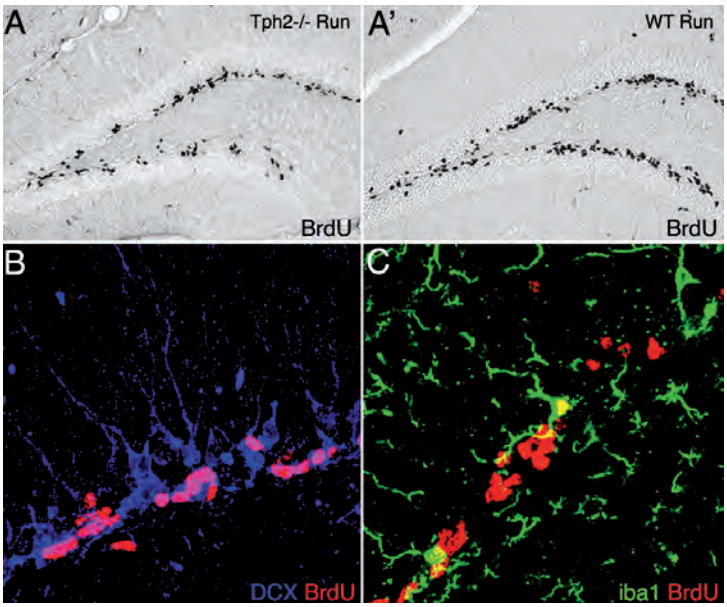


Figure 2: Serotonin is essential for exercise induced neurogenesis
BrdU labeling in the dentate gyrus in wildtype (A') and TPH2 deficient mice (A) after one week of running exercise. B, approximately 40% of newly generated cells (BrdU) express the transient immature neuronal marker Doublecortin (DCX) whereas in Tph2-deficient mice (C) increased numbers of proliferating microglia (iba1-positive) have been observed (immunohistochemistry and confocal imaging).

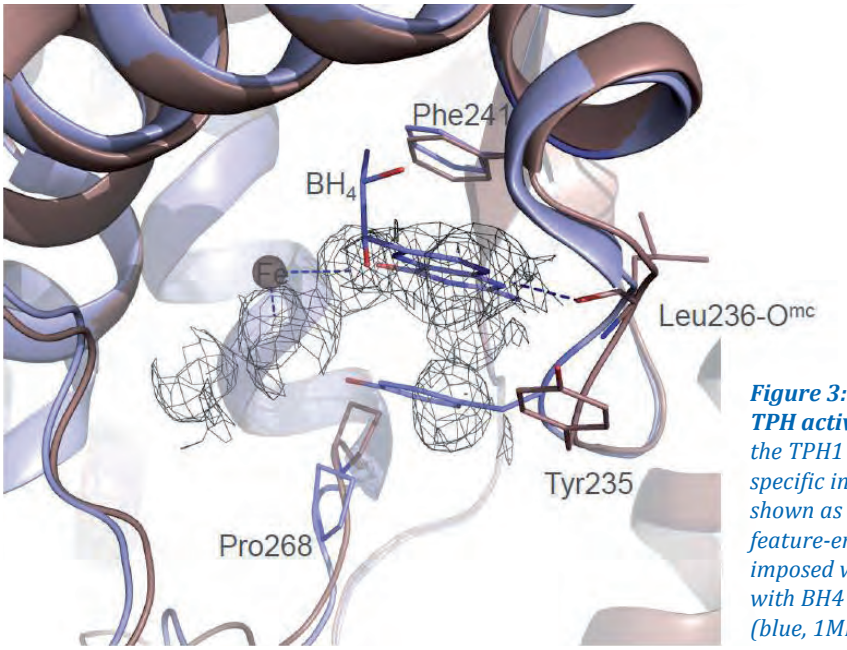


Figure 3: Novel modulators of TPH activity: *Crystal structure of the TPH1 active site with bound specific inhibitor (brown, inhibitor shown as electron density map, feature-enhanced at 0.7 σ) superimposed with a structure obtained with BH4 cosubstrate binding (blue, 1MLW.pdb).*

Furthermore, we developed protocols for the *in vitro* differentiation of embryonic stem (ES) and induced pluripotent stem (iPS) cells and transdifferentiation of mouse fibroblasts into serotonin-producing neurons and performed gene expression analysis. To evaluate if candidate genes revealed by microarray are crucial for the development of serotonergic neurons in vivo, the morpholino-based knockdown of these genes is performed in zebrafish.

Androgen receptor

Marko Bartusch, Albrecht Keil, Tina Kienitz, Meike Latz, Silke Mühlstedt, Gabin Sihn

In a collaborative work with the Charité studying gender effects in cardiac hypertrophy and failure, the group generated and characterized animal models with altered androgen receptor expression in distinct cell types of the heart and vasculature. Transgenic rats overexpressing the androgen receptor in cardiomyocytes exhibited marked alterations in cardiac rhythmicity.

Importins

Stefanie Hügel, Na Liu, Franziska Rother, Ariane Schabe, Phillip Schiele

Importins are essential components of the machinery that transports proteins into the nucleus of eukaryotic cells. In a collaborative approach with the University of Lübeck to study the physiological functions of alpha importins we have generated knockout

mice for five paralogs. The most obvious phenotype was discovered in mice lacking importin alpha7: Both sexes of these animals are infertile. The molecular basis of the male infertility is currently being analyzed. The female infertility is based on an essential function of importin alpha7 during zygotic genome activation of developing embryos. Furthermore, importin alpha7 is pivotal for influenza virus infection of cells. In parallel, proteomic analyses were performed identifying the specific import cargos of single alpha importin paralogs.

Transgenic and stem cell technology

Natalia Alenina, Ilya Chuykin, Venjamin Fishman, Alexander Krivokharchenko, Michael Lyskovikh, Markus Petermann, Elena Popova, Larissa Vilianovitch, Tianwu Qiao

The group has also a strong emphasis in the field of rat embryology and stem cell research. The rat is the preferred animal in physiological and behavioural studies. In order to obtain rat pluripotent stem cells two methodologies were applied in our group: isolation of ES cells from rat preimplantation embryos and generation of induced pluripotent stem (iPS) cells from fibroblasts upon infection with lentiviruses carrying pluripotency genes. These cells are used to explore the signalling cascades underlying mechanisms of pluripotency in the rat, to develop protocols in regenerative therapy, and to establish homologous recombination and thereby allowing gene targeting in the rat. Furthermore, transgenic rats have been produced carrying constructs, which express small interference RNAs suited to downregulate specific genes. The first target genes were the insulin receptor, apolipoprotein E, and the LDL receptor.

Patents / Patent applications

Method for diagnosing neuronal diseases and for the treatment of deficient primary hemostasis, US7049336 (B2) (23.05.2006)

Neuronally expressed tryptophan hydroxylase and its use, EP1521823 (B1) (23.05.2012)

Method for modulating, treating and/or preventing metabolic syndrome using MAS-G-protein-coupled receptor agonists, US8586054 (B2) (19.11.2013)

Selected Publications

Rousselle, A., Qadri, F., Leukel, L., Fontaine, J.-F., Rüstem, Y., Sihn, G., Bader, M., Ahluwalia, A., Duchene, J. Cxcl5 limits macrophage foam cell formation in atherosclerosis. J. Clin. Invest. 2013, 123, 1343-1347

Klempin, F., Beis, D., Mosienko, V., Kempermann, G., Bader, M., Alenina, N. Serotonin is required for exercise-induced adult hippocampal neurogenesis. J. Neurosci. 2013, 33, 8270-8275

Cui, Y., Niziolek, P.J., MacDonald, B.T., Zylstra, C.R., Alenina, N., Robinson, D.R., Zhong, Z., Matthes, S., Jacobsen, C.M., Conlon, R.A., Brommage, R., Liu, Q., Mseeh, F., Powell, D.R., Yang, Q., Zambrowicz, B., Gerrits, H., Gossen, J.A., He, X., Bader, M., Williams, B.O., Warman, M.L., Robling, A.G. LRP5 functions in bone to regulate bone mass. Nat. Med. 2011, 17, 684-691

Alenina, N., Kikic, D., Todiras, M., Mosienko, V., Qadri, F., Plehm, R., Boye, P., Vilianovich, L., Sohr, R., Tenner, K., Hörtnagl, H., Bader, M. 2009, Growth retardation and altered autonomic control in mice lacking brain serotonin. Proc. Natl. Acad. Sci. USA. 106, 10332-10337

Shmidt, T., Hampich, F., Ridders, M., Schultrich, S., Hans, V.H., Tenner, K., Vilianovich, L., Qadri, F., Alenina, N., Hartmann, E., Köhler, M., Bader, M. 2007, Normal brain development in importin alpha 5 deficient mice. Nat. Cell. Biol. 9, 1337-1338.

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Genetics and Genomics of Cardiovascular Diseases

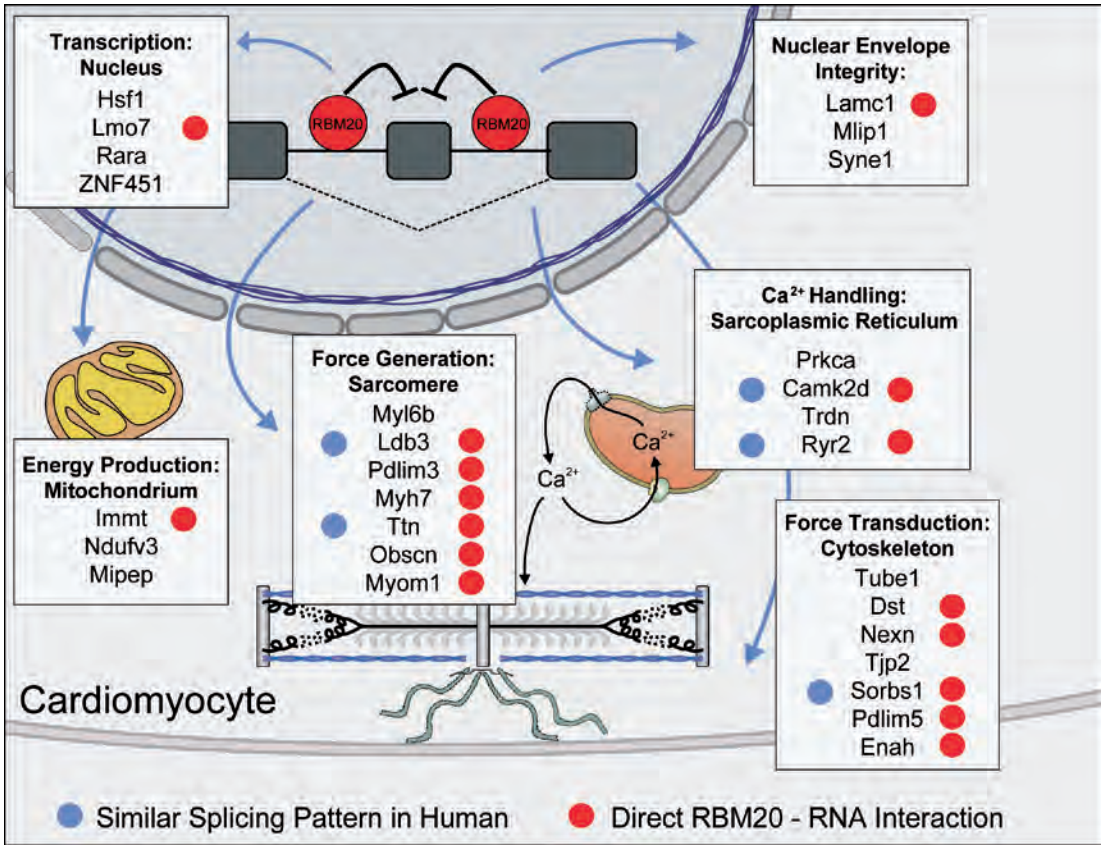
Systematic perturbations of biological networks through complete knockout or overexpression of individual genes gave insights for an understanding of the molecular basis of human diseases. The current challenge is to understand systems perturbations due to more subtle changes induced by naturally occurring DNA variation or environmental conditions that are relevant to complex diseases in human populations. We are using novel large scale technologies and advanced computation in multidisciplinary approaches to expand the systems concept and to accelerate discovery of gene networks underlying complex cardiovascular and metabolic disease phenotypes in the rat model, and to translate the findings to the molecular pathology of related human disorders. Our particular interests are inflammatory and metabolic genetic risk factors that lead to cardio- and cerebrovascular target organ damage. We use primarily the rat as a model organism to identify the major gene pathways for human cardiovascular and metabolic disorders.

Over the past years our lab has identified genes for genetically complex cardio-metabolic and inflammatory phenotypes in the rat model, all of which have been translated to advance understanding of related human phenotypes.

We have initiated the EURATRANS project, a systems biology approach to understand common cardiovascular and metabolic diseases. We have assembled a multinational consortium of world-class investigators that use technologies to generate genomic, transcriptomic, and epigenomic datasets. The EURATRANS consortium integrates these datasets in relational and dynamic models that can be used for comparative analyses to understand human gene function.

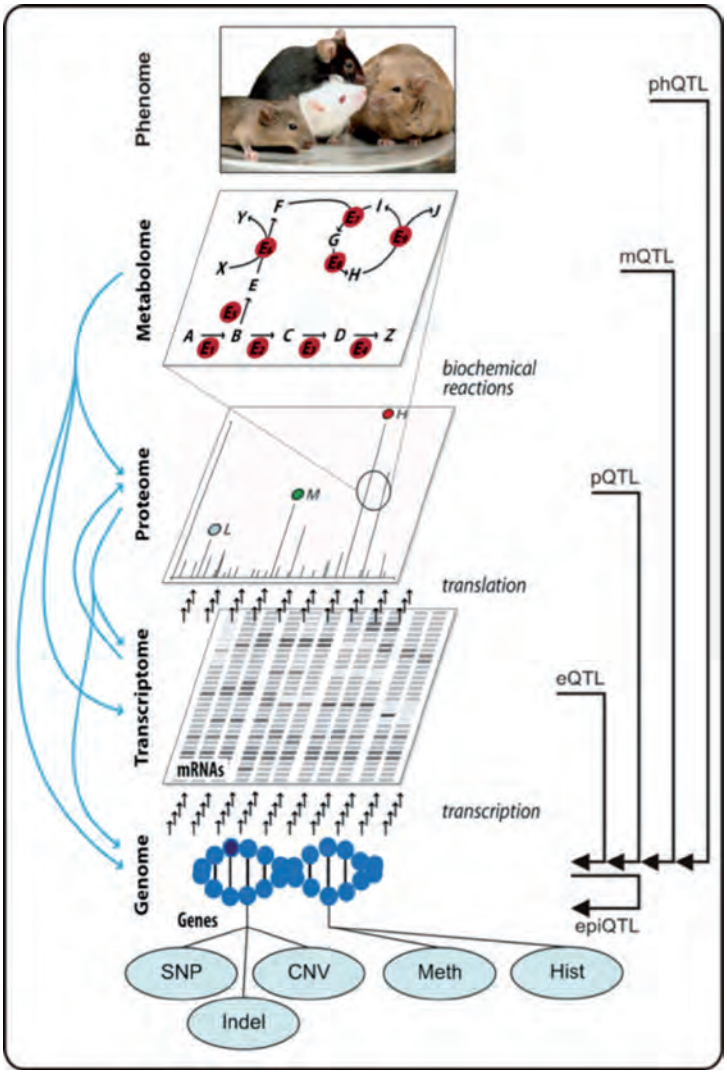
Genetic analysis of disease phenotypes in the rat.

The spontaneously hypertensive rat (SHR) is the most widely studied animal model of human hypertension and it displays a large number of other physiological and pathophysiological phenotypes, including stroke, cardiac hypertrophy and failure, and features of the human metabolic syndrome. Starting from traditional genetic linkage studies and congenic approaches, we elected to study the SHR and SHR derived substrains as a model for hypertension associated heart failure and cardiac hypertrophy. Our initial studies identified thousands of genetic control points for gene expression – or expression quantitative trait loci (eQTLs) – across the SHR genome. However, we realized that the power of genome-wide, microarray-based expression



Similar Splicing Patterns in Human (blue) and direct RBM20 - RNA Interaction (red)

profiling had more to offer. We therefore carried out extensive microarray expression studies in 7 tissues from the BXH/HXB panel of recombinant inbred (RI) strains bred from SHR and BN founders. Our collaborators and we have devised a systems approach for dissecting genetic networks systematically across the biological scale, from molecular to physiological, using segregating populations of rats. RI strains have several advantages over studies in other experimental crosses including the ability to increase trait heritability by phenotyping multiple genetically identical rats from each strain, and the ability to accumulate data from genetically stable strains over years or decades, obviating the need for de novo genotyping. A particular strength of the eQTL approach is the ability to separate genetically determined differences in gene expression into local cis-regulated control, where sequence variants controlling gene expression reside within or close to the differentially expressed gene, as compared to trans regulated control, where sequence variation resides in another gene remote from the differentially expressed gene. By identifying several of robustly mapped cis- and trans-acting expression QTLs in a model with large number of existing physiological QTLs we generated a permanent resource to test the hypothesis that genetic variation in gene expression has a key role in the molecular evolution of complex physiological and pathophysiological phenotypes that may be shared in common with similar disorders in humans. In key collaborations with T Aitman, S Cook and M Pravenec we explored the power of the eQTL approach, first to define the genetic architecture of eQTLs across multiple tissues, using new methods, and then, by correlating gene expression and linkage data, to identify genes for the individual risk factors that lead to heart failure, namely Ogn and EndoG for cardiac mass and CD36 for hypertension. Each of these genes was shown to have conserved function in human disease cohorts or tissues. In addition we were able to demonstrate that we not only could identify genes that modify individual risk factors that subsequently contribute to heart failure but that the clinically very complex endpoint heart failure is directly influenced by genetic risk factors. In addition, we generate large-scale high resolution mapping data in Heterogeneous Stock, a rat resource colony derived from 8 ancestral strains and outbred for more than 40 generations. They serve as a valuable genetic resource for simultaneous fine mapping of susceptibility genes for phenotypes in multiple disease areas, and for robust integration, at high resolution, of detailed rat genetic and physical maps. We further extend and complement the databases of gene expression and physiological phenotypes by generating transcriptome inventories, and develop new metabonomic,



Multilevel datasets will be generated in rat models for human complex diseases. We generate genomic, transcriptomic, and epigenomic datasets. We are applying quantitative metabolomic and proteomic tools to provide in-depth coverage, at multiple phenotypic levels, of the gene, cell, organ, and organism. Multiple-QTL inference models and Bayesian approaches will identify casual regulators for the disease network that will be functionally validated by in vivo and in vitro experiments. Integrating these datasets in relational and dynamic models can be used for comparative analyses to understand human gene function.

proteomic, and epigenomic datasets in RI and selected Heterogeneous Stock animals. Mapping the genetic determinants of these new phenotypes to the genome using existing high-resolution maps will permit us to identify cis and trans-regulatory control loci of these phenotypes. Using these multi-modality QTL datasets we will identify the most important pathways leading to clinically relevant phenotypes.

Integrated genomic approaches for identification of gene networks.

Our advances in rat genomics are facilitating systems-genetics approaches. We have extended the use of integrated genome-wide approaches to identify gene networks and the loci underlying their regulation. We have identified mutations in RNA binding proteins that contribute to pathological posttranscriptional regulation of an entire network of target genes that lead to cardiomyopathy and heart failure. In collaboration with Michael Gotthardt (MDC) we used genome-wide approaches in a rat strain deficient in titin splicing and found a loss-of-function mutation in RBM20 as the underlying cause for the pathological isoform expression of titin. Mutations in RBM20 in humans have previously been shown to cause dilated cardiomyopathy. We showed that RBM20 deficient rats resembled the human disease phenotype. With Cemil Özcelik (Charite) we then identified a dilative cardiomyopathy patient with an RBM20 missense mutation that led to RBM20 loss of function. Deep sequencing of the human and rat cardiac transcriptomes with RBM20 deficiency and controls

revealed an RBM20 dependent regulation of alternative splicing events. In order to understand post-transcriptional RNA processing and particularly the function of splicing factors in the heart, we, together with Markus Landthaler and Nikolaus Rajewsky, are investigating gene regulation of RBM20 in common forms of heart failure. Using PAR-CLIP we perform high resolution mapping of the binding sites of RBM20 across the transcriptome as well as those of other RNA binding proteins (RBP) interacting with RBM20. Additionally to titin we identified an RBM20 dependent network of 30 genes that is conserved between human and rat and enriched for genes previously linked to cardiomyophathy, ion-homeostasis, and sarcomere biology. We will use the resulting binding site data in knowledge-based approaches to extend our studies and to determine the impact of genomic variability on posttranscriptional modifications in humans. Our studies emphasize the importance of posttranscriptional regulation in cardiac function and provide novel mechanistic insights into the pathogenesis of human heart failure.

We are interested in epigenetic variation and gene expression in cardiovascular disease and inflammation. Histones are the main component of chromatin and undergo several posttranslational modifications including methylation. They play an important role in genome organization, stability and the control of gene expression. We will address whether there are strain-specific differences in histone modification patterns in the rat and test whether those differences are determined by allele specific sequence variants. We will use likelihood based model selection procedures to better predict the influence of genetic variability on differential gene expression. These studies shall lead to a better understanding of the interplay between epigenetic and genetic variability.

Patents / Patent applications

2010 Polynucleotides for use for Medicine

2008 Transposon mediated mutagenesis in spermatogonial stem cells

Selected Publications

Atanur SS, Diaz AG, Maratou K, Sarkis A, Rotival M, Game L, Tschannen MR, Kaisaki PJ, Otto GW, Ma MC, Keane TM, Hummel O, Saar K, Chen W, Guryev V, Gopalakrishnan K, Garrett MR, Joe B, Citterio L, Bianchi G, McBride M, Dominiczak A, Adams DJ, Serikawa T, Flicek P, Cuppen E, **Hubner N**, Petretto E, Gauguier D, Kwitek A, Jacob H, Aitman TJ. Genome Sequencing Reveals Loci under Artificial Selection that Underlie Disease Phenotypes in the Laboratory Rat. **Cell** 154(3):691-703, 2013.

Baud A, Hermsen R, Guryev V, Stridh P, Graham D, McBride MW, Foroud T, Calderari S, Diez M, Ockinger J, Beyeen AD, Gillett A, Abdelmagid N, Guerreiro-Cacais AO, Jagodic M, Tuncel J, Norin U, Beattie E, Huynh N, Miller WH, Koller DL, Alam I, Falak S, Osborne-Pellegrin M, Martinez-Membrives E, Canete T, Blazquez G, Vicens-Costa E, Mont-Cardona C, Diaz-Moran S, Tobena A, Hummel O, Zelenika D, Saar K, Patone G, Bauerfeind A, Bihoreau MT, Heinig M, Lee YA, Rintisch C, Schulz H, Wheeler DA, Worley KC, Muzny DM, Gibbs RA, Lathrop M, Lansu N, Toonen P, Ruzius FP, de Bruijn E, Hauser H, Adams DJ, Keane T, Atanur SS, Aitman TJ, Flicek P, Malinauskas T, Jones EY, Ekman D, Lopez-Aumatell R, Dominiczak AF, Johannesson M, Holmdahl R, Olsson T, Gauguier D, **Hubner N**, Fernandez-Teruel A, Cuppen E, Mott R, Flint J. Combined sequence-based and genetic mapping analysis of complex traits in outbred rats. **Nat Genet** 45(7):767-775, 2013.

Guo W*, Schafer S*, Greaser M L, Radke M H, Liss M, Govindarajan T, Maatz H, Schulz H, Lincoln S E, Parrish A M, Dauksaite V, Vakeel P, Klaassen S, Gerull B, Thierfelder L, Regitz-Zagrosek V, Hacker T A, Saupe K W, Dec G W, Ellinor P T, MacRae C A, Spallek B, Fischer R, Perrot A, Ozcelik C, Saar K, **Hubner N**, Gotthardt M. RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. **Nat Med** 18(5):766-773, 2012. *shared first author

Heinig M, Petretto E, Wallace C, Bottolo L, Rotival M, Lu H, Li Y, Sarwar R, Langley SR, Bauerfeind A, Hummel O, Lee YA, Paskas S, Rintisch C, Saar K, Cooper J, Buchan R, Gray EE, Cyster JG, Braund P, Gracey J, Krishnan U, Moore JS, Nelson CP, Pollard H, Attwood T, Crisp-Hihn A, Foad N, Jolley J, Lloyd-Jones H, Muir D, Murray E, O'Leary K, Rankin A, Sambrook J, Godfroy T, Brocheton J, Proust C, Schmitz G, Heimerl S, Lugauer I, Belz S, Gulde S, Linsel-Nitschke P, Sager H, Schroeder L, Lundmark P, Syvannen AC, Neudert J, Scholz M, Deloukas P, Gray E, Gwilliams R, Niblett D, Erdmann J, Hengstenberg C, Maouche S, Ouwehand WH, Rice CM, Samani NJ, Schunkert H, Goodall AH, Schulz H, Roider HG, Vingron M, Blankenberg S, Muenzel T, Zeller T, Szymczak S, Ziegler A, Tiret L, Smyth DJ, Pravenec M, Aitman TJ, Cambien F, Clayton D, Todd JA, **Hubner N***, Cook SA*. A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. **Nature**467:460-464, 2010. *Corresponding authors.

Joutel A, Monet-Lepretre M, Gosele C, Baron-Menguy C, Hammes A, Schmidt S, Lemaire-Carrette B, Domenga V, Schedl A, Lacombe P, **Hubner N.** Cerebrovascular dysfunction and microcirculation rarefaction precede white matter lesions in a mouse genetic model of cerebral ischemic small vessel disease. **J Clin Invest.** 120(2):433-445, 2010.

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Photo: David Ausserhofer/MDC

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Mobile DNA

Transposons (jumping genes) are segments of DNA that have the ability to move and replicate within genomes across the tree of life. Transposons offer a new model to study DNA recombination in higher organisms, as well as host-parasite interaction. Our lab tries to understand the mechanism of transposition and its regulation and to translate this knowledge to derive transposon-based genetic tools for genome manipulation or gene therapy.

Quality controls in Sleeping Beauty element (SB) transposition

I. Bilic, H. Escobar

Our understanding of how eukaryotic recombinases are working is mostly based on assuming analogies to V(D)J recombination. Besides the basic chemical reaction, the different elements have a variety of built-in regulatory mechanisms, often involving host factors, to provide specificity to the transposition reaction. One main function of such regulation is to impose quality control on transposition in the form of regulatory checkpoints. The role of these checkpoints is to avoid accumulation of incorrect reaction products in genomes.

Transposon-host interactions

A. Deveraj, V. Raghunathan, J. Rugor, M. Singh

Transposons occupy a significant portion of our genomes but the vast majority of transposons remain silent due to accumulated mutations in their genomes. The transposition of the few, active copies is strongly regulated, but this control is sensitive to environmental stress. Our results show that transposons might exist in a latent form and are able to sense developmental and environmental changes and manipulate stress signaling. Thus, cellular mechanisms directly involved in development or stress-response have a crucial role in establishing stable host-transposon co-existence.

Domesticated, transposon-derived cellular genes

A. Szvetnik, T. Raskó

Occasionally transposons are co-opted for cellular fuctions. We focus on two domesticated versions of the piggyBac transposon in the human genome. We are currently investigating the cellular function of these genes by integrated genomic and transcriptomic approaches.

Transposon mutagenesis in stem cells

I. Grabundzija, S. Bashir, A. Osiak

Transposons can be harnessed as vehicles for introducing mutations into genes. Our goal is to establish tools based on SB as well as on the *piggyBac* and *Tol2* transposon systems to manipulate vertebrate genomes in organisms. One particular application relates to loss-of-function insertional mutagenesis with the goal of generating knockout rats. The genes inactivated by transposon insertion are tagged by the transposable element, which can be used for subsequent cloning of the mutated allele. With the goal of knocking out genes implicated in disease, we carried out a pilot screen in rat spermatogonial stem cells.

Genetic background of hormone-induced breast cancer

J. Menyhért

The SB transposon is suitable for somatic mutagenesis and emerged as a new tool in cancer research. Transposon-based insertional mutagenesis screens are able to identifiy both oncogenes and tumor-suppressor genes. We are engaged in a project aiming at the discovery of novel driver mutations associated with neuroblastoma (in mice) and estrogen-induced mammary cancer (in rats).

Stress-induced transcriptional activation of transposable elements and gene expression

N. Fuchs, M. Singh, S. Narayanavari

Recent comparative genomics studies have shown that many constrained noncoding elements unique to mammals arose from mobile elements, pointing to TEs as a major creative force in the evolution of mammalian gene regulation. We recently set out to investigate the cellular functions of hyper-conserved,

TE-derived repeats in the evolution of gene regulation.

Transposons as vectors for mammalian transgenesis

A. Deveraj, S. Bashir

DNA-based transposons are natural gene delivery vehicles, and molecular reconstruction of SB is a cornerstone in applying transposition-mediated gene delivery in vertebrates, including humans. Our recently developed 100-fold hyperactive SB system, SB100X (Molecule of the Year, 2009) opened new avenues for gene delivery. Recently, we demonstrated that the SB100X system supports efficient transgenesis in various mammalian species. This seems to work with similar or improved efficacies to retro/lentiviral strategies, but it is a non-viral technique and is much simpler and safer to use. One of the important findings is that transposon-based transgenesis produces trangenics of superior quality, in that no major mosaicism is observed.

Transposons as non-viral vectors for gene therapy

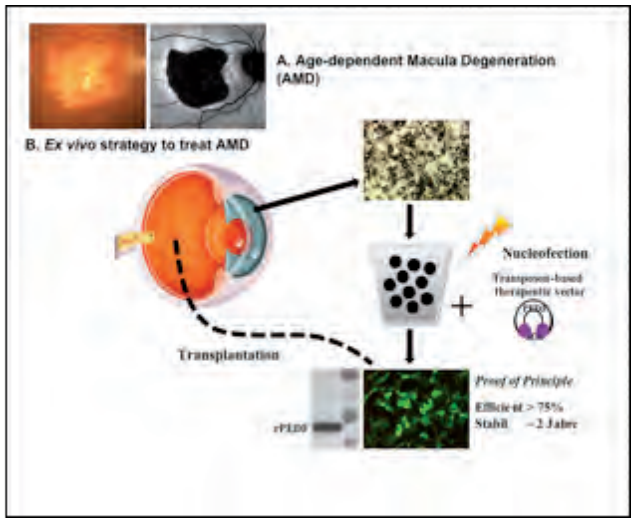
J. Wang, H. Escobar, H. Cai, A. Garcia-Perez, I. Bilic

Using the SB100X system, we are currently performing preclinical studies for gene therapy of Wiskott-Aldrich syndrome, chronic granulomatous disease (CGD), Age–related macular Degeneration (AMD) and dysferlinopathy. SB transposition occurs into chromosomes in a random manner, which is clearly undesired for human applications due to potential genotoxic effects. We succeeded in targeting SB transposition into predetermined chromosomal loci. We employed modular targeting fusion proteins, in which the module responsible for target binding can be a natural DNA-binding protein or domain, or an artificial protein. Targeted transposition could be a powerful method for safe transgene integration in human applications. The first European clinical trials, using the Sleeping Beauty transposon, will be conducted from 2015 to treat AMD (supported by FP7) and cancer patients by T cell-mediated immunotherapy (supported by BIH), respectively.

Genome-wide impact of epigenetic reset triggered by induced pluripotency

I. Grabundzija, J. Wang, N. Fuchs, E. Nagy

Due to the superior safety features of the SB-mediated gene delivery, we have established an SB100X-mediated protocol of generating induced pluripotent stem cells (iPSCs). The aim is to assess the risk of genetic destabilization inflicted by the mobilization and/or transcriptional or functional upregulation of human endogenous TEs on the genome of pluripotent stem cell lines and their differentiated derivatives.



A) Age-related macular degeneration (AMD) results from retinal pigmented epithelium degeneration. (B) The ex vivo approach is to (i) remove autologuos retinal cells, (ii) introduce the therapeutic PEDF gene with anti-angiogenic and/or neurotrophic activities into the pigment cells ex vivo by nucleofection, and (iii) re-implant the cells. Stable integration and secretion of the therapeutic PEDF (pigment epithelial derived factor) was demonstrated in >75% of the proliferating cells after several passages (followed up for 2 years).

Selected Publications

Ivics, Z, Mátés, L, Tien Yau, TY, Bashir, S, Zidek, V, Landa, V, Geurts, A, Hoffmann, O. I, Hiripi, L, Bosze, Z, Garrels, W, Kues, W. A, Pravenec, M, Rülicke, T, Izsvák, Z. 2014. Transposon-mediated germline transgenesis in rodents. Nature Prot. (tripple bac-to-back), 773-827.

Wang Y, Wang J, Devaraj, A, Singh, M, Jimenez Orgaz A, Chen JX, Selbach M, Ivics Z, Izsvák Z. 2014. Suicidal Autointegration of Sleeping Beauty and piggyBac Transposons in Eukaryotic Cells PLOS Genet. Mar 13;10(3):e1004103.

Izsvák, Z., Fröhlich, J., Grabundzija, I., Shirley, J.R., Powell, H.M., Chapman, K.M., Ivics, Z., and Hamra, F.K. (2010) Generating Knockout Rats by Sleeping Beauty Transposon Mutagenesis in Spermatogonial Stem Cells. Nature Methods 7(6):443-5.

Wang, Y., Liska, F., Gosele, C., Šedová, L., Křen, V., Křenová, D., Ivics, Z., Hubner, N. and Izsvák, Z.(2010) A Novel Active Endogenous Retrovirus Family Contributes to Genome Variability in Rat Inbred Strains. Genome Research 20(1):19-27.

Mátés, L, Chuah, MK, Belay, E, Jerchow, B, Manoj, N, Acosta-Sanchez, A, Grzela, DP, Schmitt, A, Becker, K, Matrai, J, Ma, L, Samara-Kuko, E, Gysemans, C, Pryputniewicz, D, Miskey, C, Fletcher, B, VandenDriessche, T, Ivics, Z., Izsvák, Z. (2009). Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates. Nature enet. 41(6), 753-761.

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Photo: Steffen Jänicke

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Video:
Hope for children with allergies

Molecular Genetics of Chronic Inflammation and Allergic Disease

The allergic disorders, atopic dermatitis, asthma, and hay fever, are the most common chronic diseases in childhood. The prevalence of allergic diseases has increased to epidemic dimensions over the past decades. In the industrialized countries, 25-30% of the population are affected.Our group is using genetic and genomic approaches in large study groups of affected patients and families to identify genes and genetic variants that predispose to atopic dermatitis, asthma, and chronic inflammation. Enhanced understanding of how the interaction between environmental factors, genetics, and the immune system results in inflammation will enable the development of novel diagnostic and predictive clinical tools, and of targets for innovative therapies.

Chronic inflammation has become one of the core paradigms of chronic human disease underlying numerous conditions including allergic and autoimmune disorders, cardiovascular, and neurological disease. Regardless of the mechanism of inflammation, inappropriate inflammatory responses to environmental, microbial or self antigens contribute to dysfunction and destruction of target organs. Epidemiologic data provide strong evidence for a steady rise in the incidence of diverse types of chronic inflammatory disorders in industrialized countries over the past decades, indicating that genetic variants that have evolved to arm our immune system against multiple infectious/environmental exposures now influence immune response thresholds to favor chronic inflammatory disease in the hygienic environment of modern civilization.

Our recent studies highlight the genetic overlap between risk loci for allergic disease with those for other chronic inflammatory conditions that have previously been thought to be unrelated, such as autoimmune diseases. We performed a systematic investigation of genetic markers influencing inflammatory traits as risk factors for atopic dermatitis (AD). A strong excess of AD-associated markers was observed among the selected candidate SNPs, corroborating our hypothesis. Three novel loci were significantly associated with AD, including a functional amino acid-change in the interleukin-6 receptor (IL6R Asp358Ala). Interestingly, investigation of two independent population-based birth cohorts showed that IL6R 358Ala specifically predisposes to the persistent form of AD. This variant determines the balance between the classical membrane-bound versus the soluble IL6R signaling pathways. Carriers of 358Ala had elevated serum levels of soluble IL6R with homozygote carriers showing a 2-fold increase. Moreover, we demonstrated that soluble IL6R levels were higher in AD patients than in controls. Thus, the IL6R Asp358Ala is a new risk factor for persistent AD which increases plasma levels of soluble IL6R, suggesting that targeted blocking of IL-6-transsignaling may be a novel therapeutic approach for persistent AD refractory to conventional treatments, especially in carriers of this very common variant.

The cross-disease approach was extended in the international Immunochip consortium. In this study we investigated over 24,000 individuals to evaluate 188 genetic loci involved in chronic inflammatory disorders as risk factors for atopic dermatitis. We identified four novel susceptibility loci for AD that point to genes involved in the proliferation, proliferation, migration, and functional regulation of immune cells.

Genome-wide association studies (GWAS) have provided fundamental insight into the genetic determinants of complex allergic diseases. Despite numerous findings, more than 80% of the presumed heritability remains unexplained. We participate in several international consortia enlarging study populations and increasing the power to detect allergic disease variants (e.g. GABRIEL consortium, EAGLE consortium). However, these approaches must be paralleled by different strategies to uncover the unexplained heritability. The application of current and emerging genome-sequencing technology, new techniques for analysis of gene expression and structural genome variation, as well as statistics and genome informatics will contribute to this aim on several levels.

Clinical and molecular subphenotyping.

To better understand the clinical diversity and temporal trajectory of allergic disease we have recently established a research outpatient clinic *(Hochschulambulanz)* for pediatric allergy at the Experimental and Clinical Research Center (ECRC of Charité and MDC) for the prospective evaluation of allergic sensitization patterns, disease course, and physiological evaluation in children with allergic disease. Complementing the clinical phenotype with precise molecular phenotypes may significantly enhance the identification and characterization of individual functional pathways.

Functional analysis of disease genes.

A major goal is to characterize the effects of the susceptibility genes for allergic and chronic inflammatory diseases in order to better understand the earliest events in human physiology that lead to this type of inflammatory response. We apply systematic DNA re-sequencing to extract the full variation spectrum of regulatory loci and to pinpoint culprit variants in patients and controls. Functional assays in model systems are performed to complement the molecular characterization of allergic disease risk variants. **Parent-of-origin effects.** We direct an international systematic analysis of parent-of-origin effects for eczema and asthma in four independent data sets in which genome-wide genotype data is available in complete nuclear families with allergic disease, and to replicate the findings in over 1000 independent families. Moreover, we aim to use genome-wide gene expression levels in peripheral blood mononuclear cells of a subset of 100 nuclear families with eczema as quantitative trait loci and intermediate phenotypes in our parent-of-origin analysis.

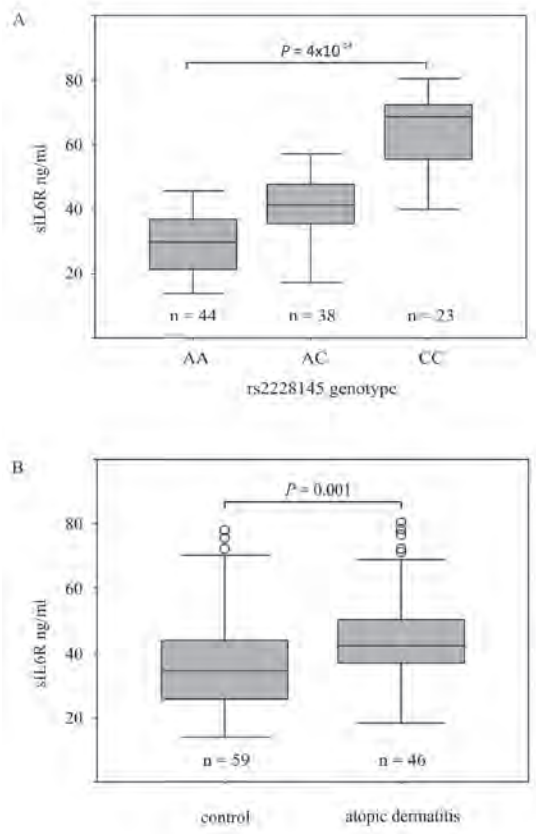


Figure 1: Soluble IL6 receptor plasma levels dependent on the functional IL6R variant (rs2228145) genotype and disease status

Box: 25th-75th percentiles; solid bar: median; whiskers: 10th-90th percentiles; circles: outliers. Number of individuals per each group is shown below each plot. A) soluble IL6R levels stratified by rs2228145 genotypes. A and C are the non-risk and risk alleles, respectively; B) soluble IL6R levels stratified by atopic dermatitis status.

Selected Publications

Ellinghaus D, Baurecht H, Esparza-Gordillo J, Rodriguez E, Matanovic A, Marenholz I, Hubner N, Schaarschmidt H, Novak N, Michel S, Maintz L, Werfel T, Meyer-Hoffert U, Hotze M, Prokisch H, Heim K, Herder C, Hirota T, Tamari M, Kubo M, Takahashi A, Nakamura Y, Tsoi LC, Stuart P, Elder JT, Sun L, Zuo X, Yang S, Zhang X, Hoffmann P, Nothen MM, Folster-Holst R, Winkelmann J, Illig T, Boehm BO, Duerr RH, Buning C, Brand S, Glas J, McAleer MA, Fahy CM, Kabesch M, Brown S, McLean WH, Irvine AD, Schreiber S, Lee YA *, Franke A *, Weidinger S *. High-density genotyping study identifies four new susceptibility loci for atopic dermatitis. Nat Genet 2013;45:808-812. * shared senior author

Esparza-Gordillo J, Schaarschmidt H, Liang L, Cookson W, Bauerfeind A, Lee-Kirsch MA, Nemat K, Henderson J, Paternoster L, Harper JI, Mangold E, Nothen MM, Ruschendorf F, Kerscher T, Marenholz I, Matanovic A, Lau S, Keil T, Bauer CP, Kurek M, Ciechanowicz A, Macek M, Franke A, Kabesch M, Hubner N, Abecasis G, Weidinger S, Moffatt M, Lee YA. A functional IL-6 receptor (IL6R) variant is a risk factor for persistent atopic dermatitis. J Allergy Clin Immunol 2013;132:371-377.

Marenholz I, Esparza-Gordillo J, Lee YA. Shared genetic determinants between eczema and other immune-related diseases. Curr Opin Allergy Clin Immunol 2013;13:478-486.

Dizier MH, Margaritte-Jeannin P, Madore AM, Esparza-Gordillo J, Moffatt M, Corda E, Monier F, Guilloud-Bataille M, Franke A, Weidinger S, Annesi-Maesano I, Just J, Pin I, Kauffmann F, Cookson W, Lee YA, Laprise C, Lathrop M, Bouzigon E, Demenais F. The ANO3/MUC15 locus is associated with eczema in families ascertained through asthma. J Allergy Clin Immunol 2012;129:1547-1553.

Scholtens S, Postma DS, Moffatt MF, Panasevich S, Granell R, Henderson AJ, Melen E, Nyberg F, Pershagen G, Jarvis D, Ramasamy A, Wjst M, Svanes C, Bouzigon E, Demenais F, Kauffmann F, Siroux V, von ME, Ege MJ, Braun-Fahrlander C, Genuneit J, Brunekreef B, Smit HA, Wijga AH, Kerkhof M, Curjuric I, Imboden M, Thun GA, Probst-Hensch N, Freidin MB, Bragina EI, Deev IA, Puzyrev VP, Daley D, Park J, Becker A, Chan-Yeung M, Kozyrskyj AL, Pare P, Marenholn I, Lau S, Keil T, Lee YA, Kabesch M, Wijmenga C, Franke L, Nolte IM, Vonk J, Kumar A, Farrall M, Cookson WO, Strachan DP, Koppelman GH, Boezen HM. Novel childhood asthma genes interact with in utero and early-life tobacco smoke exposure. J Allergy Clin Immunol 2013.

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Photo: David Ausserhofer/MDC

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Hypertension Genetics, Regulators, and Environment

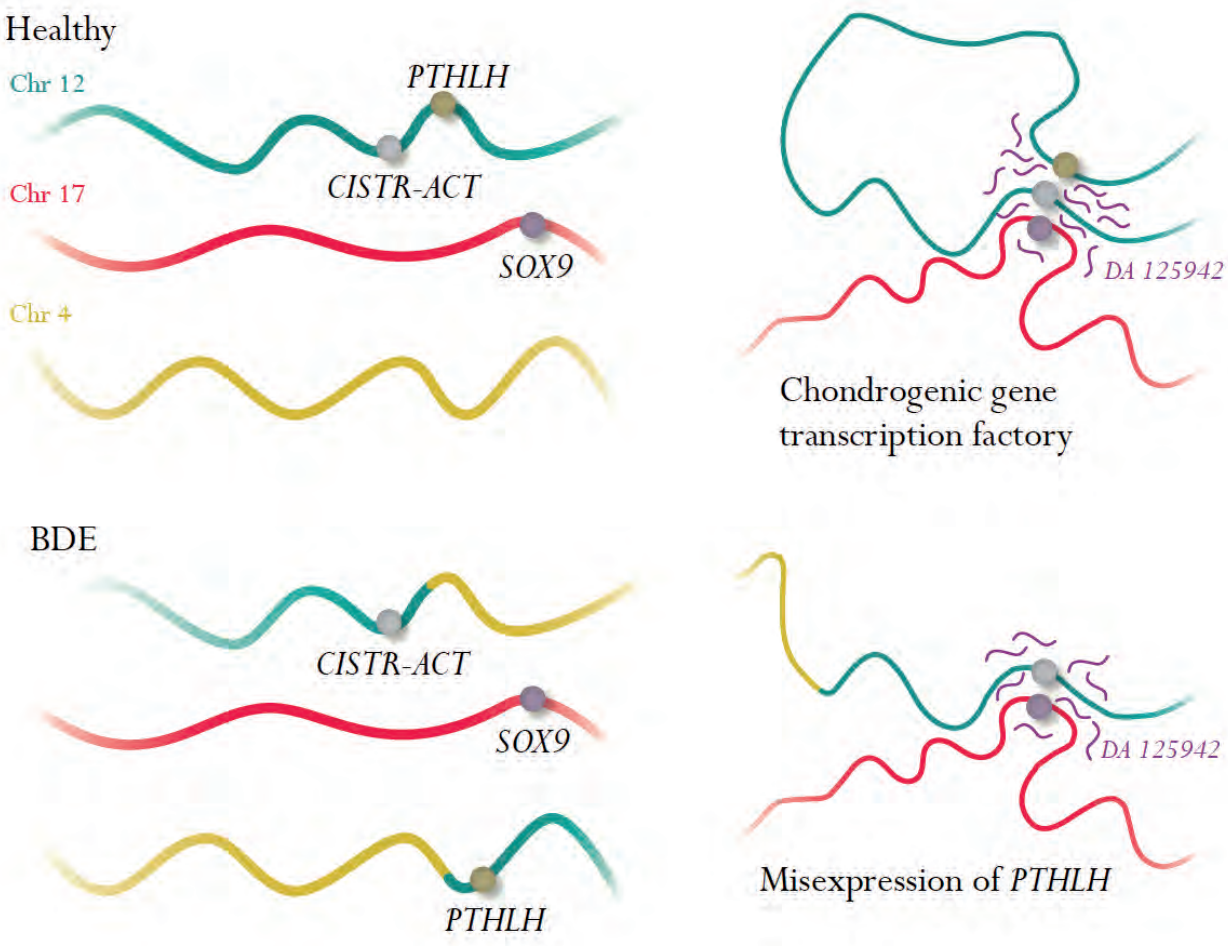


Fig. 1. *The CISTR-ACT locus (purple) and DA125942, a lncRNA transcribed from the CISTR-ACT locus, interact in cis with PTHLH and in trans with SOX9. Maass et al found that DA125942 organizes chromatin to coordinate transcription of multiple loci involved in cartilage differentiation. A chromosomal translocation found in BDE patients disrupts the ability of DA125942 to bind the PTHLH locus in cis, potentially causing premature chondrocyte differentiation at the expense of proliferation and bone elongation.*

Molecular genetics

Our goal is to reduce cardiovascular mortality; hypertension is the most important risk factor. We pursue genetic, mechanistic, and environmental influences. First, we are investigating the molecular genetics of hypertension and brachydactyly type E (BDE). The brachydactyly phenotype has led us to elucidate cis- and trans-regulatory epigenetic phenomena. Second, our group actively contributes to the elucidation of the cytochrome P450 (CYP) pathway of eicosanoid formation that has been only recently discovered to play a pivotal role in the pathogenesis of hypertension and target organ damage. We follow therapeutic strategies based on the mechanisms of CYP-eicosanoid action for the prevention of acute kidney injury and cardiac arrhythmias. Third, we are studying environmental influences particularly salt intake and subsequent sodium metabolism that we can follow with sodium magnetic resonance spectroscopy and imaging (Na-MRI) techniques.

Earlier we showed that parathyroid hormone-related peptide (PTHrP), encoded by the *PTHLH*, is a BDE gene. We recently used chromosome conformation capturing and identified a regulator on chromosome 12q that interacts in cis with *PTHLH* over a 24.4-megabase distance and in trans with the sex-determining region Y-box 9 *(SOX9)* gene on chromosome 17q. The element also harbored a long noncoding RNA (lncRNA). Silencing of the lncRNA, *PTHLH,* or *SOX9* revealed a feedback mechanism involving an expression-dependent network in humans. In the BDE patients, the human lncRNA was upregulated by the disrupted chromosomal association. Moreover, the lncRNA occupancy at the *PTHLH* locus was reduced. We identified a novel in cis- and in trans-acting DNA and lncRNA regulatory feedback element that is reciprocally regulated by coding genes. We also provided a view of how enhancers encoding lncRNAs may affect gene expression in normal development (Fig. 1.). However, our goal is to elucidate the hypertension syndrome. We have recently identified genetic mechanisms responsible for the phenotypes in this syndrome. We have identified a novel genetic mechanism that increases peripheral vascular resistance, independent of salt-reabsorptive processes. Moreover, we show that the BDE phenotype is dependent upon the same mutation (unpublished).

Eicosanoids

Eicosanoids are signaling molecules made by oxygenation of arachidonic acid (AA) and other essential omega-6 and omega-3 polyunsaturated fatty acids (n-6 and n-3 PUFAs). They exert complex control over many bodily systems, mainly in inflammation or immunity, and as second messengers of diverse growth factors and hormones throughout the cardiovascular system. Our research interest has been focused on the so-called third, and most recently discovered branch of eicosanoid formation that is catalyzed by cytochrome P450 (CYP) enzymes. CYP enzymes function as hydroxylases or epoxygenases and convert n-6 and n-3 PUFAs to unique sets of biologically active hydroxy- and epoxy-metabolites, collectively termed CYP-eicosanoids. Prominent examples include 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs).We recently showed that enhanced cardiac EET biosynthesis protects against electrical remodeling, ventricular tachyarrhythmia, and atrial fibrillation susceptibility during maladaptive cardiac hypertrophy as described in (Fig. 2). We collaborate with John R. Falck (UT Southwestern, Dallas) in developing CYP-eicosanoid-derived pharmacological approaches to prevent these disease conditions. To follow this idea from basic research to clinical phase I and phase II trials, the team founded OMEICOS Therapeutics as a spin-off company of the MDC on the BioTech-Campus Berlin-Buch *(http://www.omeicos.com)*.

Environment

Our group has worked together with Jens Titze and his group (Erlangen/Nürnberg-Vanderbilt University) to elucidate salt-related mechanisms. Briefly, we have

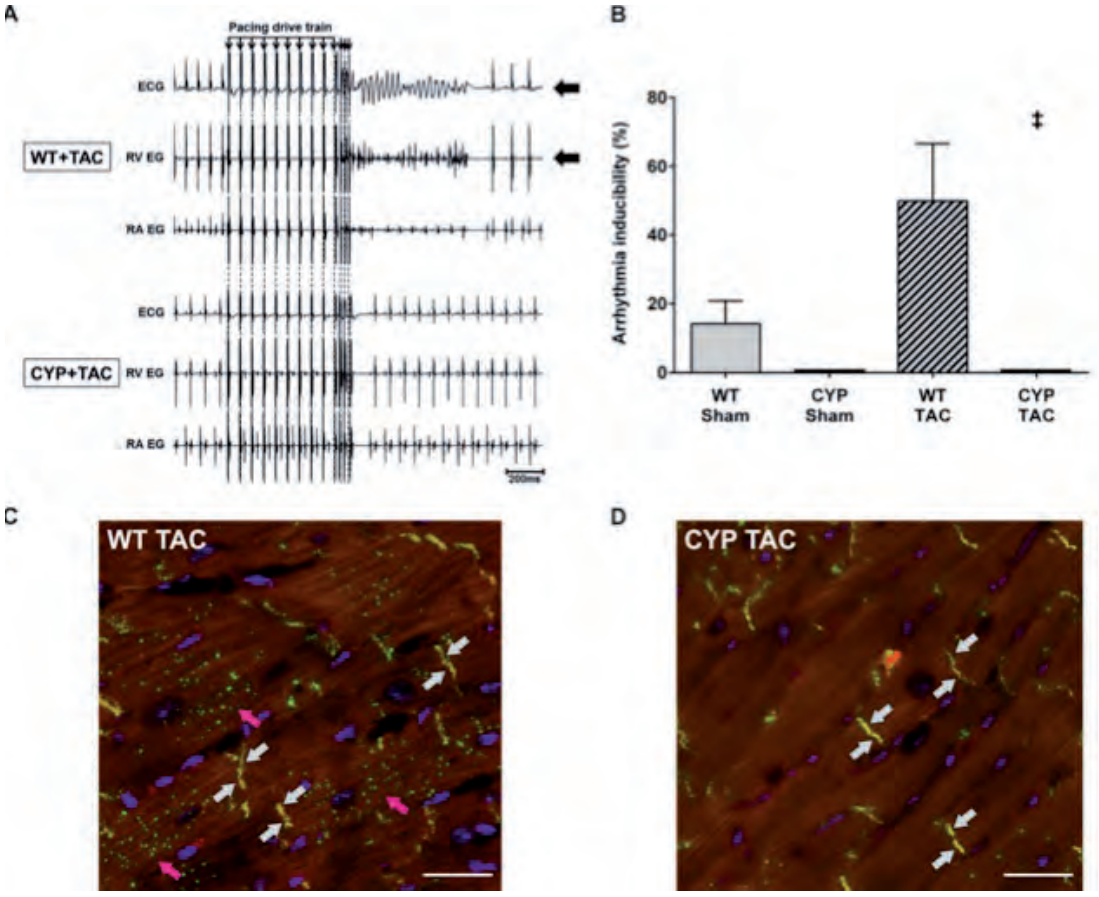


Fig. 2. *Thoracic aortic constriction (TAC) elicits ventricular-tachycardia (VT) susceptibility in wild-type (WT) mice but not in CYP2J2 (CYP) transgenic mice* **(A-B).** *Representative immunofluorescence staining of left ventricle from WT* **(C)** *and CYP2J2-TG* **(D)** *mice show normal Cx43 localization was in CYP2J2-TG mice, whereas WT mice featured TAC-induced redistribution of Cx43 to the cytoplasm and lateral borders of the cardiomyocytes (pink arrows). Nuclei were stained with DAPI (blue). Scale bar: 50 µm.*

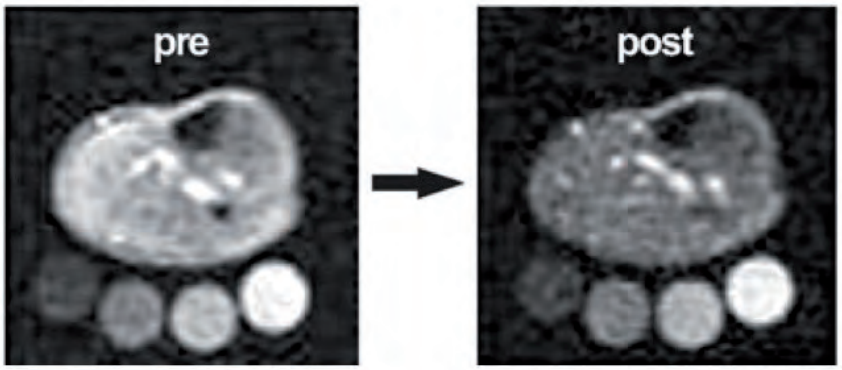


Fig. 3. *Na-MRI scan of the lower leg immediately below the knee joint in a patient with an aldosterone-secreting tumor, before (left) and after (right) removal.*

found that sodium balance follows infradian rhythms totally independent of salt intake. Total body sodium stores cycle independent of salt intake. Although high-salt intake is associated with higher blood pressure, the relationship is regulated by unknown mechanisms and not by pressure-natriuresis, as we currently understand it. We developed magnetic resonance imaging (MRI) based on sodium stimulation (Na-MRI). Na-MRI reveals hitherto fore unknown sources of sodium storage in skin and skeletal muscle.Briefly, we can "see" sodium in humans (Fig 3). The sodium is stored in soft-tissues and can be removed from stores by removal of adrenal adenomas secreting aldosterone.

Perspectives

We are optimistic. We have identified the first Mendelian hypertension related to a direct increase in peripheral vascular resistance. We are the first (and only) clinically based research group that had the courage to establish an independent company. We pursue public health (global health) goals based on genetic, cellular, animal-based, and direct translational (Na-MRI) techniques.

Selected Publications

Maass PG, Rump A, Schulz H, Stricker S, Schulze L, Platzer K, Aydin A, Tinschert S, Goldring MB, Luft FC, Bähring S. A misplaced lncRNA causes brachydactyly in humans. J Clin Invest. 2012;122:3990-4002.

Westphal C, Spallek B, Konkel A, Marko L, Qadri F, DeGraff LM, Schubert C, Bradbury JA, Regitz-Zagrosek V, Falck JR, Zeldin DC, Müller DN, Schunck WH, Fischer R. CYP2J2 overexpression protects against arrhythmia susceptibility in cardiac hypertrophy. PLoS One. 2013;8:e73490.

Wiig H, Schröder A, Neuhofer W, Jantsch J, Kopp C, Karlsen TV, Boschmann M, Goss J, Bry M, Rakova N, Dahlmann A, Brenner S, Tenstad O, Nurmi H, Mervaala E, Wagner H, Beck FX, Müller DN, Kerjaschki D, Luft FC, Harrison DG, Alitalo K, Titze J. mmune cells control skin lymphatic electrolyte homeostasis and blood pressure. J Clin Invest. 2013;123:2803-15.

Rakova N, Jüttner K, Dahlmann A, Schröder A, Linz P, Kopp C, Rauh M, Goller U, Beck L, Agureev A, Vassilieva G, Lenkova L, Johannes B, Wabel P, Moissl U, Vienken J, Gerzer R, Eckardt KU, Müller DN, Kirsch K, Morukov B, Luft FC, Titze J. Long-term space flight simulation reveals infradian rhythmicity in human Na(+) balance. Cell Metab. 2013;17:125-31.

Titze J, Dahlmann A, Lerchl K, Kopp C, Rakova N, Schröder A, Luft FC. Spooky sodium balance. Kidney Int. 2014;85:759-765.

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Eicosanoids
Dr. Wolf-Hagen Schunck
Dr. Christina Westphal
Maximilian Blum
Ramona Zummach
Christel Andrée

Environment
Natalia Rakova



Photo: David Ausserhofer/MDC

Thoralf Niendorf

Video:
How can life-threatening diseases be identified earlier?

Ultrahigh Field Magnetic Resonance (UHF-MR)

Our research concentrates on the development of MR methodology and MR technology with a focus on new ways of mapping and probing morphology, function, physiology and metabolism together with explorations into the benefits and challenges of ultrahigh field (UHF) imaging. These efforts are designed to spatially resolve and characterize (patho) physiological and biophysical processes to harmonize basic research with clinical science while balancing technical developments with clinical applications. To meet this goal small animal (9.4 T) and whole-body human (7.0 T & 3.0 T) MR scanners are put to good use at B.U.F.F. Our research is built on collaborations with partners from the Charité, the PTB, Siemens Healthcare, Bruker Biospin and several other (inter)national institutions. We form a key/integral part of major initiatives including the Helmholtz Alliance for Imaging and Curing Environmental Diseases (ICEMED), the population MRI program of the German National Cohort (GNC), the DFG research group FOR 1368 on Hemodynamics of Acute Kidney Injury, the imaging program of the German Center for Cardiovascular Research (DZHK), the EU funded FP7 project INSERT on hybrid SPECT/MR imaging, the BMBF initiative on sodium MRI (NAMRIS) and the German Ultrahigh Field Imaging (GUFI) network. Our initiatives resulted in 39 peer-reviewed publications during 2012/13, 55 abstract contributions to the 2012/13 annual meetings of the International Society of Magnetic Resonance in Medicine plus 12 (inter)national patent applications.

Technology Focus: MR Methodology & MR Technology for Ultrahigh Field MR (UHF-MR)

The sensitivity gain afforded by UHF-MR is the driving force behind our technological developments. We pioneered multi-channel radiofrequency (RF) coil technology including the design, implementation and clinical evaluation of 4-, 6-, 8-, 12- and 16-channel coil configurations tailored for cardiovascular (1), brain, ophthalmic, spine and shoulder MRI at 7.0 T **(Figure 1)**. To stay at the forefront of research we developed a modular 32 channel cardiac coil array (2). Our technology was put to good clinical use for myocardial tissue characterization (3), for uncovering subtle anatomical details of the heart and major vessels, and to drive novel ways of cardiac imaging including susceptibility mapping. Going beyond conventional proton (^{1}H) MR we are exploring MRI of sodium, fluorine, and other nuclei to gain a better insight into metabolic and (nano)molecular processes. ^{23}Na, ^{19}F, ^{1}H RF arrays designed for clinical or preclinical applications were developed to form basis of (inter)national collaborations. The group also focuses on developing novel imaging techniques (4) and ancillary hardware. These efforts include our push towards RF based hyperthermia that makes use of the traits of the short wave length regime of UHF MR. Our group developed a hybrid applicator for simultaneous targeted RF heating, temperature mapping and diagnostic imaging at 7.0 T **(Figure 2)** (5). To master the physics of even higher magnetic fields we devoted our research to numerical electromagnetic field (EMF) simulations up to B_0=30 T. Our activities culminated in our drive towards hybrid imaging (SPECT/MR, Doppler ultrasound/MR, MR/PET) to exploit the opportunities of combining anatomical, functional and metabolic imaging. MR safety is also one of our focuses, to gain a better insight into RF heating

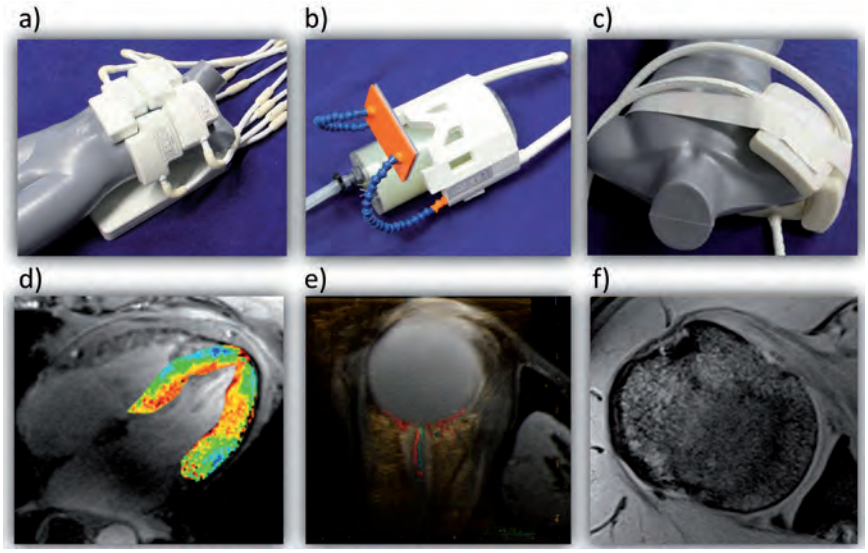


Figure 1: MR technology focus *Our research concentrates on the development of MR methodology and MR technology including pioneering novel multi-channel transmit/receive RF coil technology. Photographs of* ***(a)*** *32-channel,* ***(b)*** *6-channel and* ***(c)*** *12-channel transceiver RF coil arrays designed, implemented and clinically evaluated by our group. This enabling RF technology was put to clinical use for* ***(d)*** *high spatial resolution blood oxygenation level dependent mapping of the heart (in collaboration with A. Meloni, CNR Pisa, Italy and J. Schulz-Menger, ECRC),* ***(e)*** *for hybrid anatomical and functional ophthalmic imaging (in collaboration with O. Stachs et.al., University Rostock and S. Langner et.al., University of Greifswald) and* ***(f)*** *for MR microscopy of the shoulder (in collaboration with T. Herold et.al. Helios Clinic Berlin-Buch).*

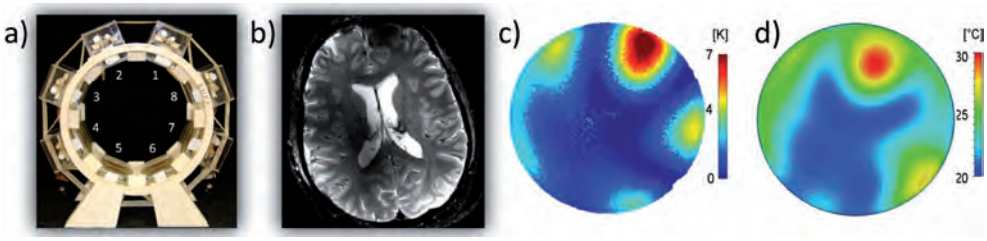


Figure 2: Next generation MR technology focus *Our group focuses on explorations into promising innovative MR applications that are not considered clinical mainstream yet. We developed ancillary hardware with the ultimate goal to enhance the capabilities for advanced diagnostics and therapy. These efforts include our push towards RF based hyperthermia that makes use of the traits of the short wave length regime of UHF MR. Our group developed a* ***(a)*** *hybrid applicator for simultaneous* ***(b)*** *diagnostic imaging,* ***(c)*** *targeted RF heating and temperature mapping at 7.0 T which was validated by* ***(d)*** *numerical temperature simulations (collaboration with P. Wust et.al. Charité and W. Hoffmann et. al., PTB).*

of stents and other medical devices, which is of paramount relevance before driving UHF-MR into the clinic.

Clinical Focus: Explorations into Cardiovascular and Neurovascular Diseases using UHF-MRI

The fields of cardiovascular and neurovascular MR are in the spotlight of our group's clinical applications-oriented research. En route to novel imaging-guided diagnostic and therapeutic approaches we strive towards the non-invasive assessment and early diagnosis of cardiac and neurovascular diseases through MR-biomarker based tissue characterization, quantitative parametric mapping, high spatial resolution and microstructural imaging of the heart **(Figure 3)**, brain and other target anatomy. These efforts will warrant an early diagnosis of pathologies (e.g. in hypertrophic cardiomyopathy), advance diagnostic imaging (e.g. of stroke, **Figure 3**), secure a differential diagnosis of pathologies (e.g. multiple sclerosis and other neuroinflammatory conditions (**Figure 3**) and drive emerging clinical applications (e.g. diagnosis of ocular masses and other diseases of the eye, orbit and optical nerve (**Figure 3**).

Experimental Focus: Quantitative Mapping and (Nano)Molecular Probing

The ultimate aim of the group is to harmonize research carried out in the area of preclinical animal imaging with that of clinical imaging. The team helps monitor glioblastoma-induced microglia attraction and tumor expansion (collaboration with H. Kettenmann,

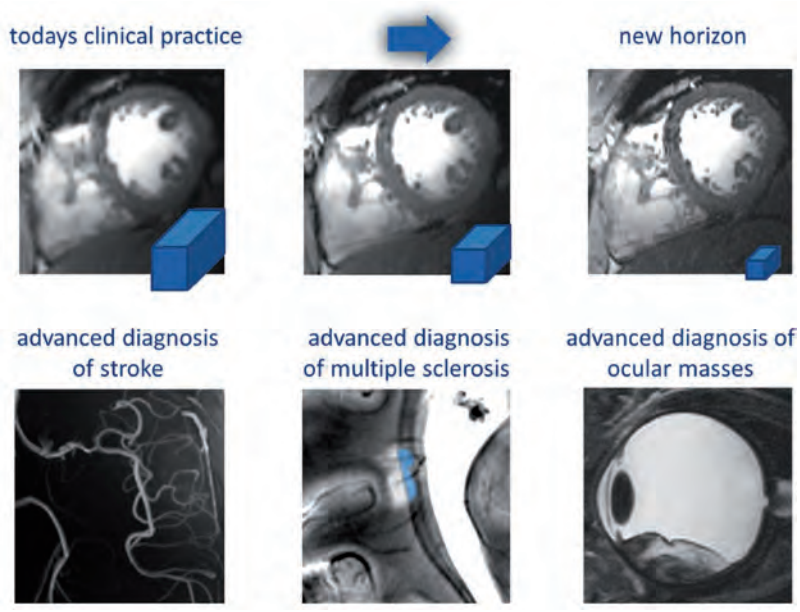


Figure 3: Clinical applications focus Top: *En route to novel imaging-guided diagnostic and therapeutic approaches we strive towards high spatial resolution and microstructural imaging of the heart. Short axis views of the heart derived from 2D CINE FLASH acquisitions of the heart at 7.0 T using a spatial resolution of* ***left****: (1.8 x 1.8 x 6) mm³,* ***middle*****: (1.4 x 1.4 4) mm³ and** ***right*****: (1.1 x 1.1 x 2.5) mm³. These results demonstrate that our novel multi-channel RF coil arrays tailored for cardiac MR at 7.0 T afford an order of magnitude spatial resolution enhancement versus standardized protocols used in today's clinical practice. This resolution gain improves the delineation of subtle anatomical details and supports microstructural imaging of the heart.* ***Bottom:*** *Our explorations into neurovascular diseases* ***left****: advance diagnostic imaging of stroke (in collaboration with J. Sobesky and V. Madai et.al., Charité) ,* ***middle*****: secure a differential diagnosis of pathologies (e.g. multiple sclerosis with the lesion marked in blue and other neuroinflammatory conditions, in collaboration with F. Paul & J. Würfel et.al., Charité) and** ***right:*** *drive emerging clinical applications (e.g. diagnosis of ocular masses and other diseases of the eye, orbit and optical nerve, collaboration with O. Stachs, University of Rostock & S. Langner et.al., University of Greifswald).*

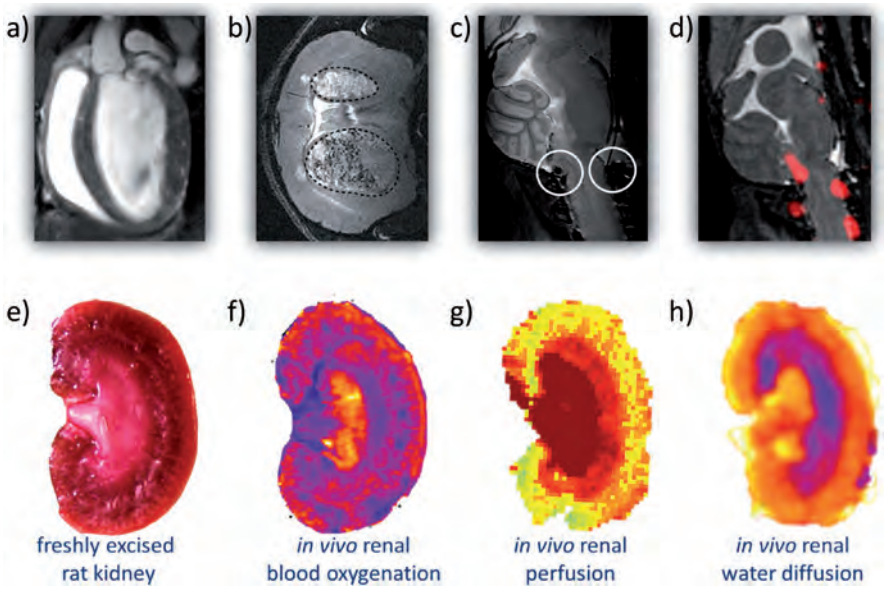


Figure 4: Experimental focus Top: *Key examples of our experimental research include* ***a)*** *non-invasive assessment of cardiac function of mice heart (collaboration with M. Bader, MDC),* ***b)*** *monitoring of brain tumour growth (collaboration with H. Kettenmann, MDC),* ***c)*** *MR microscopy of pre-symptomatic cerebellar lesions in an EAE model (collaboration with J. Millward, Charité) and* ***d)*** *tracking of ^{19}F labeled cell migration in real time with greater sensitivity in vivo together withvisualization of brain inflammation.* ***Bottom:*** *Gaining a better understanding of the mechanisms behind acute kidney injury (AKI) is in the center of our experimental research radar screen. These efforts are designed to change the landscape of non-invasive renal diagnostic imaging by benchmarking parametric MR including* ***f)*** *blood oxygenation level sensitized MRI,* ***g)*** *arterial spin labeling perfusion MRI and* ***h)*** *water diffusion weighted MRI against established quantitative but invasive physiological methods using our integrated MR-PHYSIOL setup (collaboration with E. Seeliger et.al., Charité; D. Dragun et.al., Charité ; H-W. Schunck et.al., MDC; P. Persson et.al. Charité).*

MDC) and has made major progress to study brain inflammation during the pathogenesis of autoimmune encephalomyelitis (EAE). Our know-how on fluorine (^{19}F) and physicochemical characteristics of nanoparticles is being put to good use to follow inflammatory processes and cell migration in real time with greater sensitivity in vivo. Gaining a better understanding of the mechanisms behind acute kidney injury (AKI) is in the central segment of our experimental research radar screen. These efforts are motivated by the gaps that AKI prevention and therapies still largely rely on empirical clinical knowledge and that translational approaches are still scarce. We successfully combined MRI and quantitative physiological techniques to monitor renal hemodynamics in an innovative hybrid setup (MR-PHYSIOL) with the goal to translate the diagnostic capabilities of parametric MRI from experimental research to improved clinical understanding of hemodynamics in AKI.

Future Directions

Our strategic research will continue to be formed around MR physics and drive/support collaborations and translational explorations into cardio- and neurovascular diseases. We are dedicated to throw further weight behind the solution of the remaining unmet clinical needs. To meet this goal MR technology remains to be developed and perfected. The development of next generation RF coil technology and imaging techniques will be tailored to demonstrate the clinical feasibility and value of UHF-MR. On the MR physics side we strive to extend our activities and master MR electrodynamics at ultrahigh fields by progressing further our numerical simulations and by seeking collaboration with institutions equipped with 9.4 T, 10.5 T or 11.7 T magnets, with the ultimate goal to attract an extreme field whole body MR system to B.U.F.F. We also want to extend our explorations into very promising innovative MR applications including feasibility studies on targeted drug delivery facilitated by RF heating. Following the unanimously outstanding and high praise (2013 POF evaluation) of our **Tracer Discovery and Metabolic Imaging Program** (including MR-PET imaging)proposalwe will proceed and push to turn this initiative into reality. It puts major weight behind the CMD program of the MDC and perfectly fits into the strategic objectives of the BIH. The establishment of this program will further serve to close the gap between in vitro discoveries and human (patho)physiology and will change the landscape of molecular-oriented metabolic phenotyping. It provides strong synergies between the MDC's and FMP's present strength in target discovery, screening technologies and omics with tracer development. The proposed integrated tracer discovery and metabolic imaging center provides a unique platform to form a powerful alliance that bridges the gap between molecular medicine/pharmacology and imaging-driven (pre)clinical research and provides outcome data guiding personalized medicine.

Patents / Patent applications

EP 11156004.1, WO/EP 12710456.0, WOP 11708427.7, WO/US 14/001,506, US 13/644,947, DE 102012109419.5, US 13/853,825, EP 13161942.1, EP 13161948.8, EP 13161952.0, EP 13163723.3, EP 13156151.6, DE 102013005612.8

Selected Publications

1. Niendorf T, Graessl A, Thalhammer C, Dieringer MA, Kraus O, Santoro D, Fuchs K, Hezel F, Waiczies S, Ittermann B, Winter L. Progress and promises of human cardiac magnetic resonance at ultrahigh fields: a physics perspective. J Magn Reson 2013;229:208-222.
2. Graessl A, Renz W, Hezel F, Dieringer MA, Winter L, Oezerdem C, Rieger J, Kellman P, Santoro D, Lindel TD, Frauenrath T, Pfeiffer H, Niendorf T. Modular 32-channel transceiver coil array for cardiac MRI at 7.0T. Magn Reson Med, doi: 101002/mrm24903 2013.
3. Meloni A, Hezel F, Positano V, Keilberg P, Pepe A, Lombardi M, Niendorf T. Detailing magnetic field strength dependence and segmental artifact distribution of myocardial effective transverse relaxation rate at 1.5, 3.0, and 7.0 T. Magn Reson Med, doi: 101002/mrm24856 2013.
4. Fuchs K, Hezel F, Klix S, Mekle R, Wuerfel J, Niendorf T. Simultaneous dual contrast weighting using double echo rapid acquisition with relaxation enhancement (RARE) imaging. Magn Reson Med, doi 101002/mrm25066 2013.
5. Winter L, Ozerdem C, Hoffmann W, Santoro D, Muller A, Waiczies H, Seemann R, Graessl A, Wust P, Niendorf T. Design and evaluation of a hybrid radiofrequency applicator for magnetic resonance imaging and rf induced hyperthermia: electromagnetic field simulations up to 14.0 Tesla and proof-of-concept at 7.0 Tesla. PLoS One 2013;8(4):e61661.

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Photo: David Ausserhofer/MDC

Matthias Selbach

Video:
What can we learn from counting molecules?

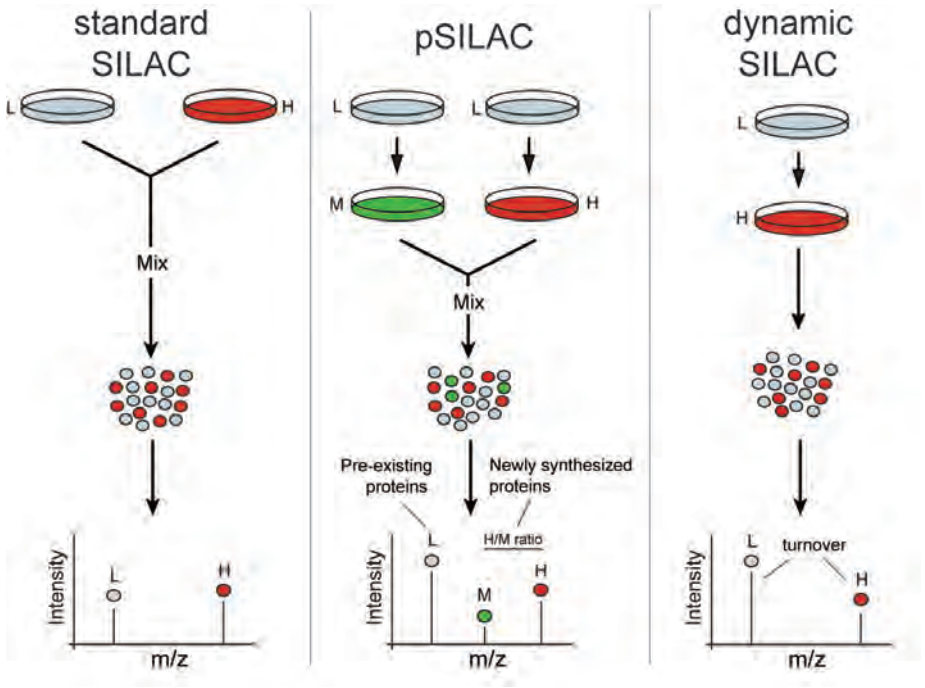
Cell Signalling and Mass Spectrometry

Understanding how the genomic information is interpreted to yield a specific phenotype is perhaps the most important question in the post-genomic era. Proteins are central players in this process: On the one hand, they are the final product of most genes. On the other hand, proteins are also directly responsible for cellular phenotypes. Proteins thus represent the central link between the genome and the phenotype. We are using quantitative mass spectrometry-based proteomics as our central technology to investigate proteome dynamics on a global scale. The lab is interested in two major questions. First, how is the genomic information processed to yield a specific proteome? Second, how do proteins that are expressed at a specific cellular condition affect the phenotype? Answering the first question requires information about protein synthesis and degradation. Systematic analysis of protein-protein interactions and posttranslational modifications can help answering the second question.

Recently developed quantitative methods make it possible to obtain precise functional information and to monitor temporal changes in the proteome by mass spectrometry. In one approach, named SILAC (for stable-isotope labelling with amino acids in cell culture), cells are differentially labelled by cultivating them in the presence of either normal or a heavy isotope–substituted amino acids. Due to their mass difference, pairs of chemically identical peptides of different stable-isotope composition can be distinguished in a mass spectrometer. The ratio of intensities for such peptide pairs accurately reflects the abundance ratio for the corresponding proteins. SILAC can be used to quantify differences in steady-state protein levels (Fig. 1, left). In addition, pulsed SILAC can be employed to measure differences in protein synthesis (Fig. 1, middle). Finally, dynamic SILAC can reveal protein turnover (Fig. 1, right). Combined with high throughput mass spectrometry, the different variants of SILAC enable quantification of different aspects of proteome dynamics on a global scale.

Gene expression control

The four fundamental cellular processes involved in gene expression are transcription, mRNA degradation, translation and protein degradation. Each of these four steps is controlled by gene-regulatory events. So far, little is known about how the combined effect of all regulatory events shapes gene expression. The fundamental question of how genomic information is processed to obtain a specific cellular proteome is therefore still largely unknown. We are using metabolic pulse labelling approaches to comprehensively quantify gene expression. Protein turnover can be quantified using dynamic SILAC. Similarly, newly synthesized RNA can be labelled with nucleoside analogues. Mass spectrometry and next generation sequencing (in collaboration with the lab of Wei Chen) then allows us to quantify the absolute abundance of mRNAs and proteins and their half-lives in parallel. These data can then be used to calculate synthesis rates of mRNAs and proteins by mathematical modeling (collaboration with the group of Jana Wolf). Our results indicate that gene expression in mouse fibroblasts is predominantly controlled at the level of translation (Schwanhausser, 2011). Consistently, we find that translation is actively regulated during Schwann cell development in vivo (collaboration with the lab of Carmen Birchmeier, Sheean et al., 2014). Currently, we are investigating how the different levels of



Different variants of SILAC for the analysis of cellular proteome dynamics. *Cells are metabolically labelled with light (L), medium-heavy (M) or heavy (H) stable isotope-encoded amino acids. Differentially labelled peptides can be distinguished by mass spectrometry. The different variants of SILAC allow global quantification of different aspects of proteome dynamics.*

gene expression change upon perturbation. In addition, we are using pulsed SILAC (pSILAC) to directly quantify changes in protein synthesis. For example, we are studying the impact of microRNAs and RNA-binding proteins on protein production.

Protein-protein interaction

Proteins typically interact with other proteins to exert a specific cellular function. Identifying interaction partners therefore provides direct insights into protein function and can reveal disease mechanisms. We are using quantitative mass spectrometry and to analyze protein-protein interactions (Paul et al., 2011). This approach has two unique advantages. First, accurate quantification allows us to distinguish specific interaction partners from background contaminants with very high confidence. Second, quantification reveals how interactions change in response to perturbation. We are using this method to study how disease-associated mutations and cell signaling events affect protein-protein interactions. We are also investigating how cellular interaction partners of influenza A virus proteins differ between strains with different pathogenic potential.

In vivo quantitative proteomics: The SILAC zoo

Cell culture-based experiments cannot recapitulate all of the complex interactions among different cell types and tissues that occur *in vivo*. Small animal models such as worms and fruit flies are attractive alternatives that are extensively used in many areas of biomedical research, especially in genetics and development. We have extended the SILAC technology to *Caenorhabditis elegans* (collaboration with the lab of Nikolaus Rajewsky) and *Drosophila melanogaster* (Sury et al., 2010; Grun et al., 2014). Currently, we are using these models to study protein-protein interactions *in vivo*.

Selected Publications

Sheean, M.E., McShane, E., Cheret, C., ..., Selbach, M., and Birchmeier, C. (2014). Activation of MAPK overrides the termination of myelin growth and replaces Nrg1/ErbB3 signals during Schwann cell development and myelination. Genes Dev 28, 290-303.

Grun, D., Kirchner, M., Thierfelder, N., Stoeckius, M., Selbach, M., and Rajewsky, N. (2014). Conservation of mRNA and Protein Expression during Development of C. elegans. Cell Rep.

Paul, F.E., Hosp, F., and Selbach, M. (2011). Analyzing protein-protein interactions by quantitative mass spectrometry. Methods 54, 387-395.

Schwanhausser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., and Selbach, M. (2011). Global quantification of mammalian gene expression control. Nature 473, 337-342.

Sury, M.D., Chen, J.X., and Selbach, M. (2010). The SILAC fly allows for accurate protein quantification in vivo. Mol Cell Proteomics 9, 2173-2183.

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Photo: David Ausserhofer/MDC

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Cardiovascular Molecular Genetics

Inherited cardiomyopathies, programming of the fetal heart and the cardiac role of nuclear receptors represent the major foci of our research group. Arrhythmogenic right ventricular cardiomyopathy, a difficult-to-diagnose inherited heart muscle disorder, originates from mutated desmosomal proteins ultimately causing severe structural alterations, dangerous ventricular arrhythmias and heart failure. Correct fetal programming is known as a prerequisite for normal function in adulthood but little is known on how this is achieved at the molecular level. A second focus of our research is, therefore, the molecular dissection of adequate programming steps in the fetal heart. Interfering with cardiac mitochondrial function during development is our tool to shed light on this issue. Whether or not liver X receptor agonists are potential targets for the treatment of metabolic, inflammatory and cardiovascular diseases is the third topic of our interest.

Arrhythmogenic right ventricular cardiomyopathy

Arnd Heuser

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heritable primary cardiac muscle disorder characterized by the replacement of cardiomyocytes with fibrofatty tissue, predominantly in the right ventricle (RV). The resulting disruption of normal myocardial architecture can lead to RV dysfunction, life-threatening arrhythmias, and sudden cardiac death. Disease causing mutations have been identified mainly in genes encoding desmosomal proteins.

We were the first to identify mutations in plakophilin2 (PKP2) and desmocollin2 (DSC2) as a cause of autosomal dominant ARVC. PKP2 mutations account for a significant proportion of ARVC cases (10-45%). PKP2 and DSC2 are components of the desmosomal intercellular junction complex (Figure 1) known to be essential for maintaining tissue integrity and increasingly implicated in cell signaling. While the involvement of multiple desmosomal protein in ARVC has led to speculations about the sensitivity of the myocardium to mechanical disruption, the true pathogenic mechanisms of ARVC causing mutations are largely unknown.

Desmosomes and adherence junctions build a special “mixed” type of junction in the intercalated disc, the area composita. Mature desmosomes with extended area composita structures in the heart form in a late, primarily postnatal process. Accordingly, we hypothesize that desmosomes

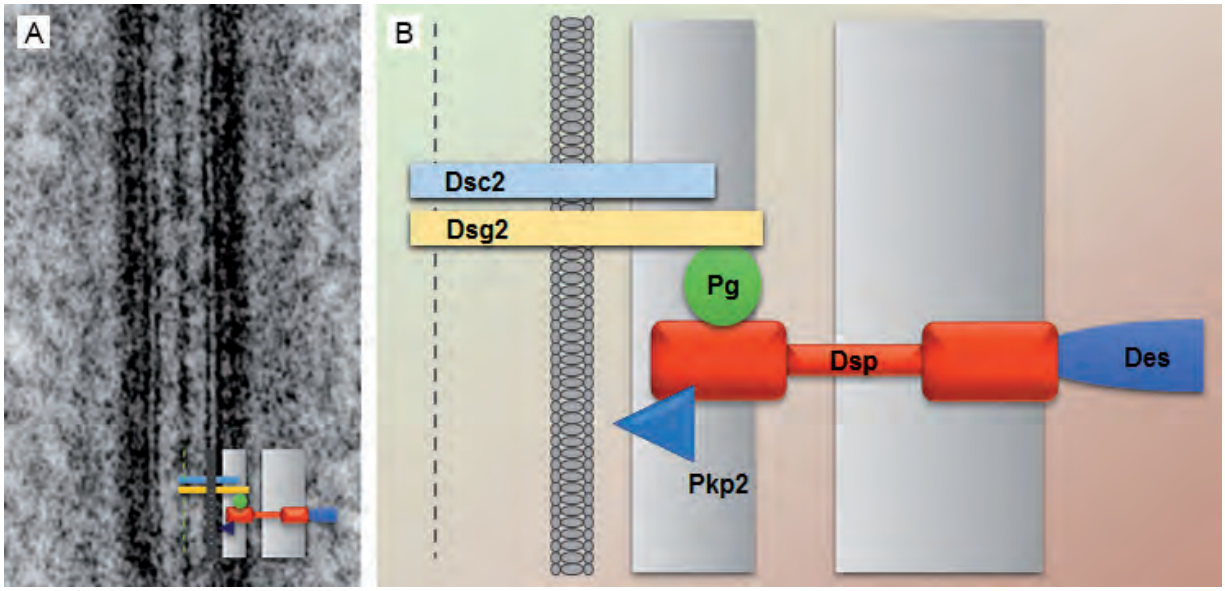


Figure 1: Structural organization of desmosomes. *(A) Electron micrograph of desmosomes from murine heart. (B) The schematic drawing shows desmosomal cadherins (DSG, desmoglein; DSC, desmocollin); the armadillo-family members plakoglobin (PG) and the plakophilins (PKP); the desmosomal linker-protein desmoplakin (DSP); and the intermediate filament-binding desmin (DES).*

play an essential role for the mechanical integrity of cardiomyocytes in the adult heart. To test this hypothesis we investigate cardiac-specific knockout models of different desmosomal components in adult mice. Ablation of the essential desmosomal proteins Pkp2 and Dsp lead to substantial structural changes at the intercalated disc. Ongoing work defines the adhesive and signaling properties of these proteins in the heart. This should shed light on the pathogenicity of ARVC, thereby facilitating the development of new diagnostic procedures and therapeutic strategies.

Embryonic heart regeneration, fetal programming and cardiac organ size control

Jörg Drenckhahn

The prenatal heart possesses an impressive growth plasticity in response to both endogenous as well as environmental or maternal conditions. In this regard, our recent findings have shown that the embryonic murine heart has a remarkable regenerative capacity. We have inactivated the X-linked gene encoding Holocytochrome c synthase (Hccs), an enzyme essential for normal function of the mitochondrial electron transport chain, specifically in the developing mouse heart. Loss of Hccs activity results in cellular energy starvation causing disturbed cardiomyocyte differentiation and ultimately cellular degeneration. In contrast to the observed mid-gestational lethality of hemizygous *Hccs* knockout (KO) males, heterozygous females appeared normal during postnatal life with surprisingly few clusters of defective myocardium, considering an expected mosaic of affected and normal cardiomyocytes as a result of random X chromosomal inactivation. However, analyses of heterozygous female ($Hccs^{+/-}$) embryos revealed the expected 50:50 ratio of Hccs deficient to normal cardiac cells at mid-gestation with a progressive reduction in disease tissue to 10% prior to birth. We could show that this significant change is accounted for by increased proliferation of remaining healthy cardiac cells. These data reveal a previously unrecognised but impressive regenerative capacity of the mid-gestational heart that can compensate for an effective loss of at least 50% of cardiac tissue to enable formation of a functional heart at birth.

Yet despite this regeneration, hearts of neonatal *Hccs*$^{+/-}$ females are hypoplastic at birth evident as a reduction in heart weight, thinning of left ventricular (LV) walls and a significantly reduced number of cardiomyocytes compared to littermate controls. The reduction in LV mass, however, normalizes until adulthood and this could be attributed to an increase in cardiomyocyte size (hypertrophy) in *Hccs*$^{+/-}$ females (while proliferation rates are unaltered suggesting that cardiomyocyte number is not normalized). These data suggest the activation of compensatory cardiac growth mechanisms in the postnatal heart after disturbed heart development. Investigation of the underlying molecular mechanisms revealed alterations of several metabolic regulators, such as mTOR and Ampk, as well as activation of ER (endoplasmic reticulum) stress and amino acid metabolism. mTOR, an important regulator of translation and cell growth, is intensively studied in the context of pathological as well as physiological cardiac hypertrophy in adulthood, however, surprisingly little is known about its relevance for the prenatal heart. Therefore, we developed a rapamycin treatment protocol in pregnant mice to efficiently inhibit mTOR in fetuses during late gestation. Prenatal rapamycin treatment results in general growth restriction, evident as a 12% reduction in body weight at birth. Importantly, heart weight was reduced by 33% (Figure 2), resulting in a reduced heart weight to body weight ratio in rapamycin versus vehicle treated neonates. Other organs were less affected, highlighting the importance of mTOR for perinatal cardiac growth. Current projects intent to further study the role of mTOR during embryonic and fetal heart development using genetic models of heart conditional inactivation of mTOR components in mice.

To assess the importance of amino acid availability for cardiac growth and organ size control, we kept pregnant mice on a low protein diet throughout pregnancy. Such amino acid restriction did not alter developmental patterning or growth of the embryonic heart, but results in reduced heart size in adult mice if kept on a low protein diet after birth. The latter is the result of reduced cardiomyocyte size, which however did not affect cardiac contractility. In conclusion, sufficient amino acid availability appears to be primarily important for postnatal hypertrophic but not prenatal proliferative growth of the heart. Current projects intend to thoroughly characterize the role of amino acid availability during the transition from pre- to postnatal cardiac growth pattern.

Unfavorable intrauterine growth conditions have been shown to predispose the heart for cardiac disease later in life, a process referred to as fetal programming. To study the consequences of embryonic heart regeneration for the adult heart we subjected *Hccs*$^{+/-}$ females to chronic Angiotensin II infusion. Although Ang II did not affect cardiac function when compared to controls, it dramatically altered cardiomyocyte growth kinetics as well as molecular stress response resulting in hyperactivation of JAK/STAT3 signaling in H*ccs*$^{+/-}$ hearts. This clearly confirmed that disturbing prenatal cardiac development renders the response of the postnatal heart to pathological conditions. Additionally, pharmacological inhibition of JAK/STAT3 during Ang II stress impairs the compensatory growth response in *Hccs*$^{+/-}$ hearts resulting in contractile dysfunction when compared to controls. Based on these data we propose a new model linking fetal programming to growth and organ size control in the adult and ageing heart.

Role of Nuclear Receptors in Cardiac Metabolism and Hypertrophy

Florian Blaschke

Cardiac hypertrophy leading to heart failure is a major cause of morbidity and mortality worldwide. The reason why cardiac dilatation and failure eventually occur are unknown although alterations in energy status and metabolism are proposed to play an important role. An understanding of the role of cardiac energy metabolism and hypertrophic gene expression in the pathology of cardiomyopathy may consequently lead to more effective novel therapeutic strategies for the prevention and treatment of heart failure. We are currently elucidating the role of nuclear receptors in cardiac metabolism and hypertrophy and charac-

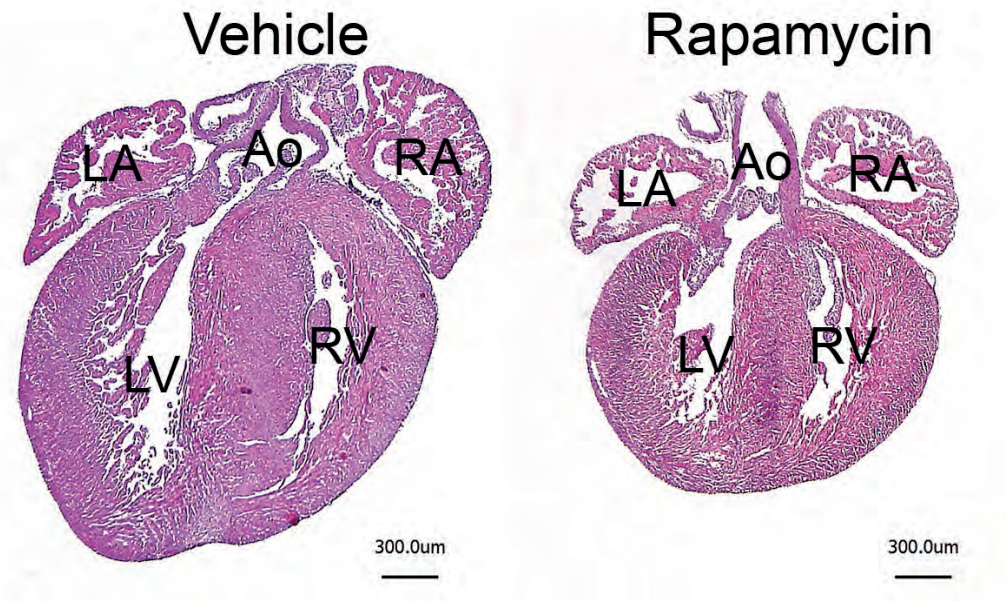


Figure 2: Prenatal mTOR inhibition results in impaired cardiac growth and reduced heart size at birth. *Pregnant female mice were treated with either vehicle or the mTOR inhibitor rapamycin during late gestation. Newborn hearts of the resulting offspring were embedded in paraffin, sectioned and stained with hematoxylin & eosin (H&E). Overview images show a striking reduction in heart size in rapamycin compared to vehicle treated newborns. LV/RV = left and right ventricle, LA/RA = left and right atrium, Ao = aorta (scale bar = 300 µm)*

terize the molecular mechanism(s) utilized to regulate gene expression involved in cardiomyocyte growth and energy metabolism.

Given their pleiotropic effects of nuclear receptors and their activation by specific ligands in lipid and glucose homeostasis, cardiac energy balance and regulation of inflammatory gene expression, new drugs targeting nuclear receptor are emerging as promising therapeutics for the treatment of diabetes, obesity, atherosclerosis, restenosis and heart failure.

Selected Publications

Heuser A, Plovie ER, Ellinor PT, Grossmann, KS, Shin JT, Wichter T, Basson CT, Lerman BB, Sasse-Klaassen S, Thierfelder L, MacRae CA, Gerull B. (2006). Mutant desmocollin-2 causes arrhythmogenic right ventricular cardiomyopathy (ARVC). Am J Hum Genet. Dec;79 (6):1081-8.

Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, Lerman BB, Markowitz SM, Ellinor PT, Macrae CA, Peters S, Grossmann KS, Michely B, Sasse-Klaassen S, Birchmeier W, Dietz R, Breithardt G, Schulze-Bahr E, Thierfelder L.(2004). Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. [Corrigendum in: Nat Genet. 2005 Jan;37(1):106]. Nat. Genet. 36, 1162-1164.

Kirchner F, Schuetz A, Boldt LH, Martens K, Dittmar G, Haverkamp W, Thierfelder L, Heinemann U, Gerull B.(2012). Molecular insights into arrhythmogenic right ventricular cardiomyopathy caused by plakophilin-2 missense mutations. Circ Cardiovasc Genet. 5(4):400-11.

Drenckhahn JD, Schwarz QP, Gray S, Laskowski A, Kiriazis H, Ming Z, Harvey RP, Du XJ, Thorburn DR, Cox TC (2008). Compensatory growth of healthy cardiac cells in the presence of diseased cells restores tissue homeostasis during heart development. Developmental Cell 15 (4): 521-533.

Blaschke F, Takata Y, Caglayan E, Collins A, Tontonoz P, Hsueh WA, Tangirala RK. A nuclear receptor corepressor-dependent pathway mediates suppression of cytokine-induced C-reactive protein gene expression by liver X receptor. Circ Res. 2006 Dec 8;99(12):e88-99. Epub 2006 Nov 16.

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Photo: David Ausserhofer/MDC

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Molecular Epidemiology

The Molecular Epidemiology Group studies at the molecular level the relationship between lifestyle, diet, genetic, metabolic, and environmental factors with risk and outcome of chronic diseases in human populations. The focus is on biomarkers that have the potential for accurate and precise assessment of exposure, intermediary effects, and early disease development. Our aims are to contribute to the understanding of disease etiology and pathogenesis in humans, to allow a more precise prediction of diseases, and to improve the identification of high risk individuals as well as of the quantification of the effect of interventions, thus envisioning a reduction of chronic disease risk through targeted prevention.

Metabolic factors and cancer risk

Obesity is associated with metabolic diseases, including cardiovascular diseases and cancer *(Aleksandrova, Front Biosci (Elite Ed) 2013)*. Abdominal obesity contributes to chronic disease risk and mortality beyond general obesity *(Pischon, N Engl J Med 2008)*, and is associated with abnormal glucose metabolism, elevated blood pressure, dyslipidemia, and type 2 diabetes mellitus, which cluster within the Metabolic Syndrome. Within the EPIC study we found that the Metabolic Syndrome is associated with a higher risk of colon cancer and that this association is largely accounted for by abdominal obesity and abnormal glucose metabolism *(Aleksandrova, Cancer Prev Res 2011)*. Abdominal obesity is mostly accounted for by visceral fat, which is metabolically active and secretes a variety of cytokines and hormones. We investigated the role of the adipocyte-derived hormones adiponectin and leptin *(Aleksandrova, Curr Nutr Rep. 2013).* Adiponectin improves insulin sensitivity and has antiangiogenic properties. Leptin is a regulator of food intake and energy balance, and may also induce tumor angiogenesis, reduce apoptosis, and promote cell growth and migration. In EPIC we found no significant relationship between leptin concentrations and colon cancer risk, but a strong inverse relationship for the soluble leptin receptor (sOB-R), which was statistically significant even after adjustment for leptin *(Aleksandrova, Cancer Res 2012).* In addition, we found high concentrations of non-high molecular weight (HMW) adiponectin associated with lower colorectal cancer risk *(Aleksandrova, Carcinogenesis 2012).* Out of 9 biomarkers, the association between abdominal obesity and colon cancer risk was accounted mostly for by 3 markers, HDL-C, non-HMW adiponectin, and sOB-R *(Aleksandrova, Int J Cancer. 2014)*. We are currently investigating the association between fetuin-a (a liver biomarker related to insulin resistance) and risk of colorectal cancer (*Nimptsch).*

The liver is central in human metabolism, and obesity is closely associated with non-alcoholic steatosis, steatohepatitis, liver cirrhosis, and liver cancer. We studied prospectively the association of inflammatory and metabolic biomarkers with risks of liver and bilary tract cancers *(Aleksandrova, Hepatology 2014)*. After multivariable adjustment, we found higher concentrations of CRP, IL-6, C-peptide and non-HMW adiponectin associated with higher risk of he-

patocellular carcinoma, suggesting that elevated levels of biomarkers of inflammation and hyperinsulinemia are associated with higher risk of HCC, independent of obesity and established liver cancer risk factors.

Metabolic factors and diabetes risk

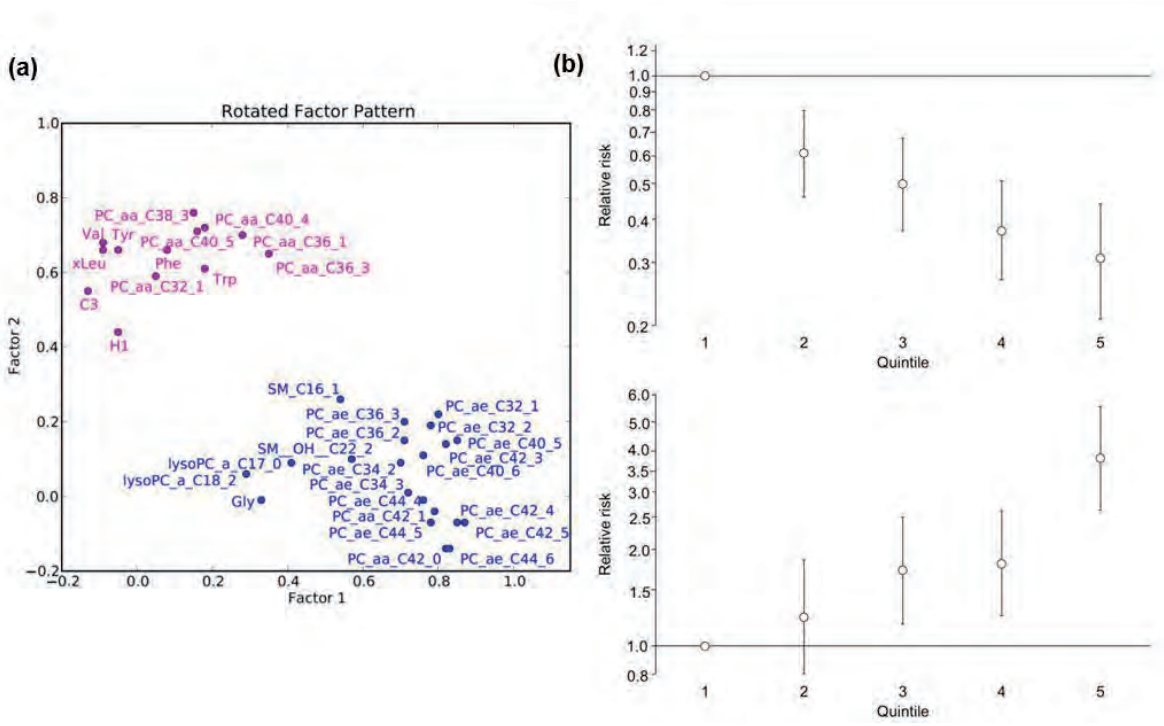
A number of biomarkers have been proposed for the estimation of type 2 diabetes (T2D) risk. We previously showed that out of 7 biomarkers the highest relative contribution to risk of T2D was observed for adiponectin, followed by GGT and HDL-C, and less so for CRP *(Montonen, Eur J Epidemiol 2011).* Design and advancement of high-throughput techniques determined the emergence of metabolomics, which is the simultaneous study of numerous low-molecular weight compounds. We prospectively investigated the association between 163 serum metabolites measured by targeted metabolomics and risk of T2D in EPIC-Potsdam *(Floegel, Diabetes 2013)*. Serum hexose; phenylalanine; and diacyl-phosphatidylcholines C32:1, C36:1, C38:3, and C40:5 were independently associated with increased risk of T2D and serum glycine; sphingomyelin C16:1; acyl-alkyl-phosphatidylcholines C34:3, C40:6, C42:5, C44:4, and C44:5; and lysophosphatidylcholine C18:2 with decreased risk. Variance of the metabolites was largely explained by two metabolite factors in opposing directions. The data indicate that metabolic alterations, including sugar metabolites, amino acids, and choline-containing phospholipids, are associated early on with a higher risk of T2D.

Diet, nutrition, lifestyle

High consumption of red meat is associated with increased risk of colorectal cancer, cardiovascular disease and type 2 diabetes, whereas high intake of whole grains is related to reduced risk. Consumption of red meat may increase insulin resistance, oxidative stress and inflammation, whereas intake of whole grains may provide protection by improving insulin sensitivity, lowering insulin and glucose levels, and by inhibiting inflammation and oxidative stress. We examined the association of red meat and whole-grain bread consumption with biomarkers related to metabolic pathways in the EPIC-Potsdam study *(Montonen, Eur J Nutr. 2013)*. Higher consumption of whole-grain bread was significantly associated with lower levels of GGT, ALT and hs-CRP, whereas higher consumption of red meat was significantly associated with higher levels of GGT and CRP when adjusted for potential confounding factors related to lifestyle and diet. Further adjustment for BMI and waist circumference attenuated the association between red meat and CRP (P=0.19). These data suggest that high consumption of whole-grain bread is related to lower levels of GGT, ALT and hs-CRP, whereas high consumption of red meat is associated with higher circulating levels of GGT and CRP.

Consumption of red meat is the major source of iron intake in humans and related to T2D risk. Impaired glucose metabolism and diabetes mellitus are common clinical manifestations of iron overload in patients with haemochromatosis, a disorder of abnormal iron absorption resulting in the progressive accumulation of iron in inner organs. We examined the association of body iron stores with risk of type 2 diabetes in EPIC-Potsdam (*Montonen, Diabetologia 2012).* After multivariable adjustment, higher serum ferritin concentrations were associated with a higher risk of type 2 diabetes. No significant association was observed for soluble transferrin receptor. These results support the hypothesis that higher iron stores below the level of haemochromatosis are associated with risk of type 2 diabetes.

Diet and physical activity are among the main determinants for risk of major chronic diseases in Western civilizations. Yet, it is less clear what determines diet and physical activity. As part of the BMBF funded Knowledge Hub on the Determinants of Diet and Physical Activity Choice (DEDIPAC), which is part of the European Joint Programme Initiative "A Healthy Diet for a Healthy Life" we are investigating determinants of diet and physical activity. In addition, within the ActivE study, we are in-



Serum metabolites and risk of type 2 diabetes. *(a) metabolite factors identified by principal component analysis; (b) association of metabolite factors with risk of incident type 2 diabetes. Source: Floegel et al, Diabetes 2013*

vestigating determinants of physical activity related energy expenditure. Further, we examine associations of adipose tissue gene expression profiles with metabolism, nutrition, diet, and lifestyle.

Genetic Variation in metabolic factors and risk of chronic diseases

We previously observed a positive association between circulating levels of the inflammatory marker CRP and risk of colorectal cancer (CRC) in the EPIC study *(Aleksandrova, Am J Epidemiol 2010)*. In a further step, we applied the concept of Mendelian Randomization (MR) to investigate whether genetic variants associated with lifelong differences in CRP concentrations are associated with risk of CRC. We observed that CRP genetic variability leading to raised CRP concentrations was associated with higher risk of CRC (Nimptsch), supporting the hypothesis that circulating CRP may play a causal role in the etiology of this cancer. MR studies will also be conducted to investigate potential causality of the adipokines adiponectin, leptin, sOB-R and fetuin-a.

The National Cohort

With the aim to develop new strategies for risk assessment, early detection, and prevention of major chronic diseases, a network of German research institutions, including the MDC, has started a large prospective cohort to be used as a common, national resource for studies on risk factors and etiologic mechanisms of major diseases in the German population. The National Cohort will include 200,000 participants, to be recruited through a network of 18 study centers, organized in 8 clusters throughout Germany. At each center, a random sample of the general population will be drawn within strata of age and sex. All participants will be invited to the study centers to take part in physical and medical examinations, collection of biomaterials, personal interviews, and to fill in questionnaires. All participants will be re-invited for a second examination five years after baseline recruitment. Participants will be re-contacted every 2-3 years and asked to fill in short questionnaires about changes in lifestyle and other characteristics and about the occurrence of selected, major diseases. In parallel, a mortality follow-up and systematic record linkage with existing disease registries will be performed periodically. Biomaterials will be stored in a centralized biobank and in decentralized storages. Within this initiative, the Molecular Epidemiology Group coordinates the Cluster Berlin-Brandenburg, which consists of the MDC, the Charité-University Medical Center Berlin, and the German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE). The Cluster com-

prises three study centers, which will recruit 30,000 participants into the National Cohort.

Pretests for the National Cohort

A major foundation for the planning and preparation of the National Cohort was the implementation of pretests to assess the feasibility of methods for recruitment of study participants and for assessment of study data. In addition, the pretests formed the basis to build up recruitment centers and hire qualified personnel. Within these pretests, we have recruited 420 participants from Berlin and Brandenburg. The participants underwent a base set of detailed interviews and physical examination that were standardized across all study centers. In addition, we have conducted feasibility studies in collaboration with partner institutions, to evaluate methods to assess physical activity, physical fitness, nutrition, and oral health. Further, we have developed methods for shipment and long-term storage of biomaterials. During the pretests, blood samples have been collected from 1576 participants from the three study centers of the Cluster Berlin-Brandenburg, and are being stored at the MDC. Furthermore a study protocols for the assessment of body measures using a 3-dimensional body surface scanner has been developed and evaluated.

MRI measurements within the National Cohort

Magnetic resonance imaging (MRI) permits comprehensive imaging using highly standardized protocols without ionizing radiation. In prospective settings, it offers the opportunity to study the relevance of preclinical morphological changes for chronic disease risk. In the German National Cohort, whole-body 3T MRI measurements will be taken from 30.000 participants. At the MDC's Berlin Ultrahigh Field Facility (B.U.F.F.), the Molecular Epidemiology Group, the Experimental Ultrahigh-Field MR group, and the Cardiac MRI group will conduct MRI measurements of 6.000 study participants from the three Berlin-Brandenburg study centers of the German National Cohort. The measurements will be the cornerstone for future analysis on exposure disease relationships.

Selected Publications

Aleksandrova K, Boeing H, Nothlings U, Jenab M, Fedirko V, Kaaks R, Lukanova A, Trichopoulou A, Trichopoulos D, Boffetta P, Trepo E, Westhpal S, Duarte-Salles T, Stepien M, Overvad K, Tjonneland A, Halkjaer J, Boutron-Ruault MC, Dossus L, Racine A, Lagiou P, Bamia C, Benetou V, Agnoli C, Palli D, Panico S, Tumino R, Vineis P, Bueno-de-Mesquita B, Peeters PH, Gram IT, Lund E, Weiderpass E, Quiros JR, Agudo A, Sanchez MJ, Gavrila D, Barricarte A, Dorronsoro M, Ohlsson B, Lindkvist B, Johansson A, Sund M, Khaw KT, Wareham N, Travis RC, Riboli E, Pischon T. Inflammatory and metabolic biomarkers and risk of liver and bilary tract cancer. Hepatology 2014.

Aleksandrova K, Drogan D, Boeing H, Jenab M, Bas Bueno-de-Mesquita H, Jansen E, van Duijnhoven FJ, Rinaldi S, Fedirko V, Romieu I, Kaaks R, Riboli E, Gunter MJ, Romaguera D, Westhpal S, Overvad K, Tjonneland A, Halkjaer J, Boutron-Ruault MC, Clavel-Chapelon F, Lukanova A, Trichopoulou A, Trichopoulos D, Vidalis P, Panico S, Agnoli C, Palli D, Tumino R, Vineis P, Buckland G, Sanchez-Cruz JJ, Dorronsoro M, Diaz MJ, Barricarte A, Ramon Quiros J, Peeters PH, May AM, Hallmans G, Palmqvist R, Crowe FL, Khaw KT, Wareham N, Pischon T. Adiposity, mediating biomarkers and risk of colon cancer in the European Prospective investigation into cancer and nutrition study. Int J Cancer 2014;134:612-21.

Floegel A, Stefan N, Yu Z, Muhlenbruch K, Drogan D, Joost HG, Fritsche A, Haring HU, Hrabe de Angelis M, Peters A, Roden M, Prehn C, Wang-Sattler R, Illig T, Schulze MB, Adamski J, Boeing H, Pischon T. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes 2013;62:639-48.

Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven FJ, Rinaldi S, Fedirko V, Romieu I, Riboli E, Gunter MJ, Westphal S, Overvad K, Tjonneland A, Halkjaer J, Racine A, Boutron-Ruault MC, Clavel-Chapelon F, Kaaks R, Lukanova A, Trichopoulou A, Lagiou P, Trichopoulos D, Mattiello A, Pala V, Palli D, Tumino R, Vineis P, Buckland G, Sanchez MJ, Amiano P, Huerta JM, Barricarte A, Menendez V, Peeters PH, Soderberg S, Palmqvist R, Allen NE, Crowe FL, Khaw KT, Wareham N, Pischon T. Leptin and soluble leptin receptor in risk of colorectal cancer in the European Prospective Investigation into Cancer and Nutrition cohort. Cancer Res 2012;72:5328-37.

Montonen J, Boeing H, Steffen A, Lehmann R, Fritsche A, Joost HG, Schulze MB, Pischon T. Body iron stores and risk of type 2 diabetes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Diabetologia. 2012 Oct;55(10):2613-21.

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Photo: David Ausserhofer/MDC

Matthew N. Poy

microRNA and Molecular Mechanisms of Metabolic Diseases

Type II diabetes has finally become recognized as a major challenge to global health. It is imperative to improve our understanding of the molecular mechanisms behind this disorder and develop new drug therapies. The pathophysiology of diabetes is undoubtedly complex, typically characterized by hyperglycemia resulting from varying states of insulin resistance and impaired b-cell function. Oftentimes, the failure to regulate circulating blood glucose levels is a consequence of the inability to produce sufficient amounts of insulin by the pancreatic β-cells. In our group, we focus on fundamental pathways regulating glucose metabolism and how altered pancreatic islet physiology contributes to metabolic disorders such as type 2 diabetes.

To date, numerous counter-regulatory mechanisms have been characterized in the cell illustrating the dynamic nature of the intracellular environment. Among the more recently discovered is the microRNA (miRNA) pathway and as of this writing, 2578 mature human miRNAs have been annotated in the miRBase database which now catalogs 206 different plant and animal species. Importantly, since their identification in C.elegans, a canonical pathway has now been identified to describe how miRNAs are processed and incorporated into Argonaute-containing complexes to maintain their effect on gene expression. Many insights into the functional role of miRNAs have been recently made, improving our understanding of how these small RNAs integrate into the already complex landscape of regulating gene expression. Most notably, based on a number of miRNA knockout mouse models showing subtle phenotypes under steady state conditions, a hypothesis has gradually emerged suggesting the miRNA pathway contributes to cellular stress responses. Moreover, these observations are further supported by our recent study focusing on the role of Argonaute2 (Ago2) in the compensatory proliferation of the pancreatic β-cell during insulin resistance. These recent findings culminate over 10 years since our initial study which identified miR-375, a miRNA now established among the highest expressed sequences in this cell type.

The very first loss and gain-of-function studies revealed this miRNA as a negative regulator of insulin release via the direct targeting of the gene myotrophin. The role of miR-375 in insulin release was further confirmed in vivo using the total mouse knockout (375KO). Furthermore, this model facilitated the identification of several additional targets of miR-375 including Elavl4/HuD, Cadm1, Gphn, and Rasd1. Moreover, 375KO mice exhibited hyperglycemia and decreased β-cell mass, and these effects were further exacerbated after crossing the knockout onto the leptin-deficient ob/ob background. This result indicated that miR-375 plays an essential role in the compensatory β-cell proliferation induced by insulin-resistance in ob/ob mice. While the precise causes are not understood, insulin resistance is one of the most common metabolic stress conditions and a consequence of chronic over-nutrition. Moreover, our recent observations showing loss of Ago2 also blocked proliferation during insulin resistance further supports

a role for the miRNA pathway in mediating cellular stress responses. Importantly, we showed that Ago2 is up-regulated in the pancreatic islets of both genetic and diet-induced models of insulin resistance and obesity as a result of the silencing of miR-184. Furthermore, the inverse relationship between Ago2 and miR-184 was confirmed in islets of human subjects further underlining the relevance of studying β-cell function in these mouse models. Of note, miR-184 was shown to target the gene Slc25a22, a mitochondrial glutamate transporter, indicating miRNAs may regulate many stages of the canonical pathway in the β-cell leading to insulin secretion.

While miR-184 has been shown to potently regulate both growth and secretion of the β-cell, it remains to be determined how changes in insulin sensitivity as shown in the models of obesity may contribute to the direct regulation and function of all miRNAs in this cell type. Meanwhile, it is unclear whether miRNA function in the β-cell may also change in response to alterations within its metabolic environment such as levels of extracellular nutrients (glucose, amino acids or fatty acids), signaling hormones, neurotransmitters, or perturbations in cell-to-cell contact with neighboring endocrine, endothelial, mesenchymal, or neuronal cells. Moreover, key transcriptional regulators of miRNAs in the β-cell as well as RNA-binding proteins that may specifically bind miR-184 and target it for degradation remain unidentified. It is unclear how the additional abundant β-cell miRNAs may coordinately target genes to promote insulin release as well as facilitate compensatory expansion of the β-cell as metabolic demand increases. While miR-184 was the most significantly regulated miRNA in the islets of insulin-resistant mice, additional miRNAs have been shown to participate in the β-cell growth using human islet cells8. It will presumably be determined that numerous miRNAs act in a concerted fashion to regulate multiple targets that mediate cell growth and the continual recruitment of insulin-containing granules to the plasma membrane. Interestingly, it is still not known whether the same miRNAs perform an identical regulatory function in different cell types. As Ago2-associated sequences such as miR-375 are expressed in several unique tissues such as the pituitary and adrenal gland, it is unclear how this miRNA may regulate growth and secretion in these other neuroendocrine cell types.

In summary, in light of recent findings identifying components of the RNAi machinery in the nucleus including Ago2, Dicer, and GW182, it appears only a matter of time before new mechanistic insights into the role of this pathway are discovered. While the identification of direct targets of the miRNAs and their biological significance remains to be studied in greater detail, the role of small RNAs in the nucleus also remains to be described. The story of non-coding RNAs and their functional role in the pancreatic β-cell is far from complete. In an exciting time of rapidly evolving technologies, the next 10 years are certain to bring clarity to this expanding narrative.

Selected Publications

Tattikota, SG., Rathjen, T., Hausser, J., Pandey, AK., Wessels, HH., Esguerra, JLS., McAnulty, SJ., Musahl, A., You, X., Chen, W., Herrera, P., O'Carroll, D., Eliasson, L., Zavolan, M., and Poy, MN. Argonaute2 mediates compensatory expansion of the pancreatic β-cell. (2014) Cell Metabolism. 19(1):122-34.

Tattikota, SG., Sury, MD., Rathjen, T., Wessels, HH., Pandey, AK., You, X., Becker, C., Chen, W., Selbach, M., and Poy, MN. (2013) Argonaute2 regulates the pancreatic β-cell secretome. Molecular & Cellular Proteomics. 12, 1214–1225.

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Photo: David Ausserhofer/MDC

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Genetics of Metabolic and Reproductive Disorders

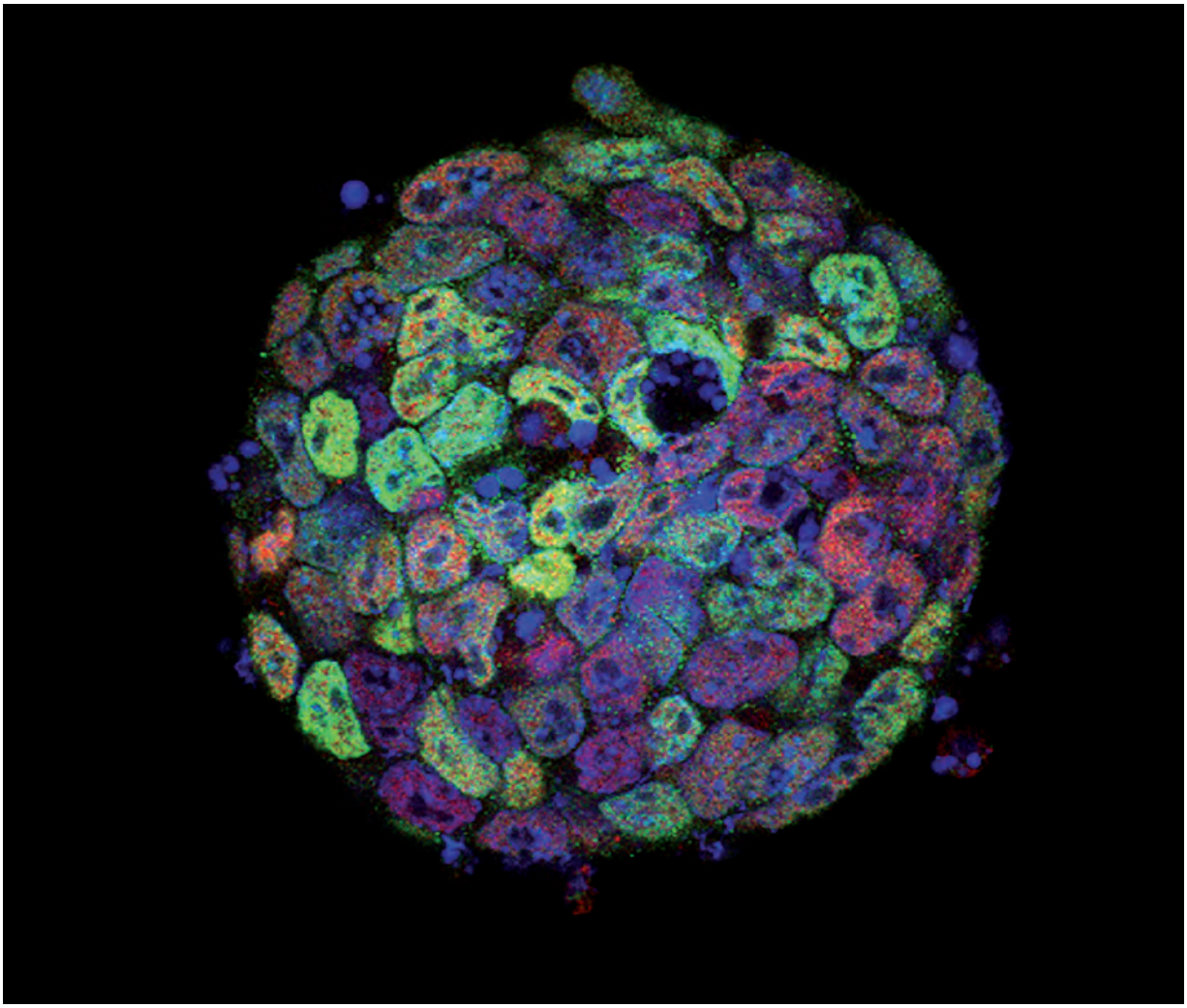
Our research is focused on the genetic regulation of metabolic and developmental processes. Transcriptional regulators are at the center of our investigations. In particular, we have started to look how metabolic changes influence the epigenetic landscape modulating the transcriptional response to meet the challenge during adaptation to novel environmental conditions. With a series of mouse models for human diseases that we created over the last years, we are now in a position to dissect even complicated physiological questions at the organismal level.

Stem cells, transcription factors and epigenetic regulation

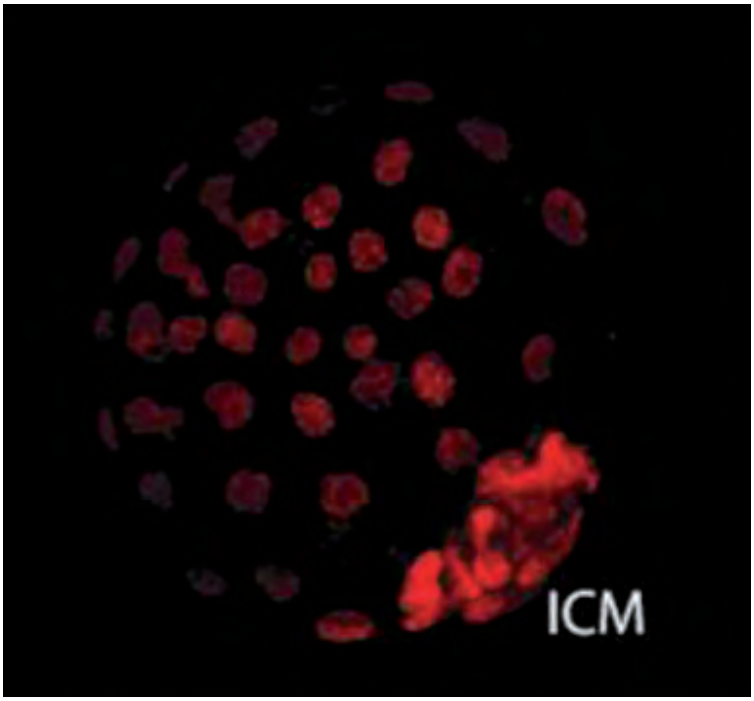
Stem/progenitor cell populations constitute the basic building units from which organs and whole organisms are created.

We identified the transcriptional regulator SALL4 as a key player that is required to maintain the pluripotency state of embryonic stem cells. SALL4 is highly expressed in the inner cell mass (ICM) of the blastocyst that gives rise to the embryo and the primitive endoderm. During the course of our studies we identified Rbpjk, the effector of the Notch pathway, as a Sall4 interaction partner. Subsequent purification of the Rbpjk protein complex confirmed the Sall4/Rbpjk interaction. Furthermore, ChIP-seq analysis for Sall4 and Rbpjk confirmed occupancy of common DNA loci throughout the genome. Furthermore, we could show that Sall4 directly regulates transcriptional activity of a Notch/Rbpkj response elements providing a possible molecular explanation for the observed neural differentiation of ES cells upon Sall4 depletion.

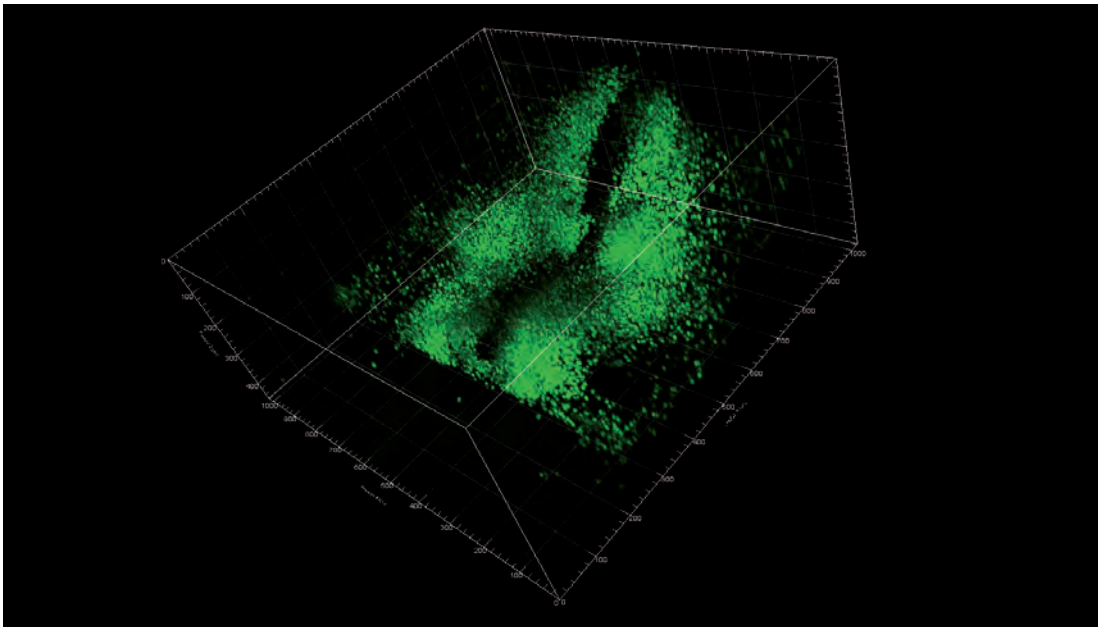
At the moment, our interests are focused on how embryonic stem cell pluripotency and self-renewal is maintained at the molecular and biochemical level. Answers to these fundamental questions should enhance our understanding of epigenetic modifications underlying embryonic stem cell pluripotency and self-renewal and provide novel insights into the process of reprogramming of somatic genomes into a pluripotent or alternative cell fate.



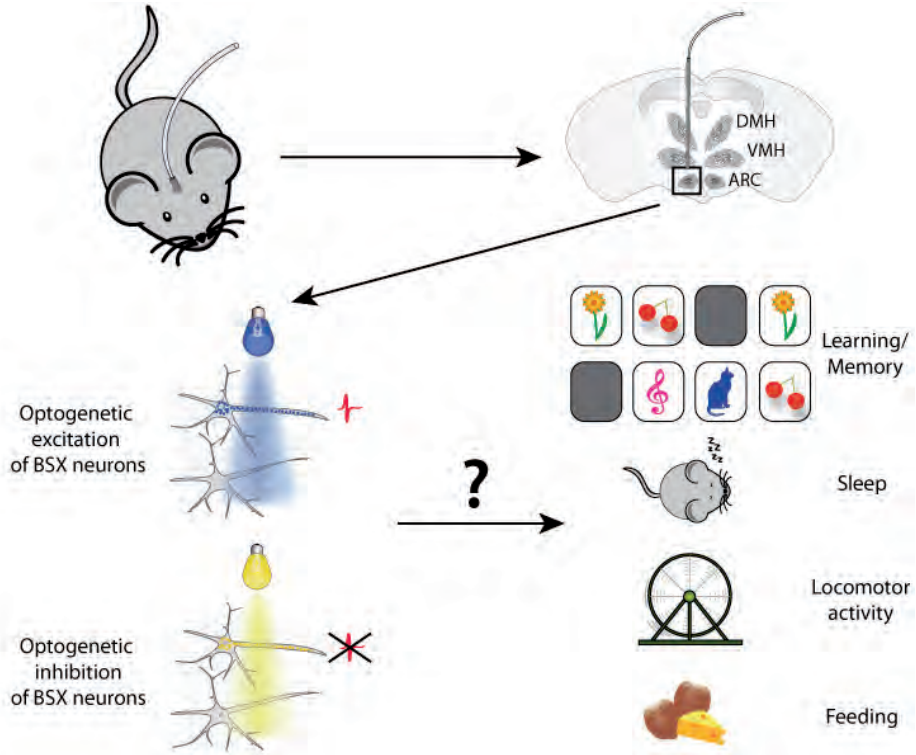
Murine embryonic stem cells stained with antibodies against DNMT3 and Nanog.



Sall4 staining of the inner cell mass (ICM) in a mouse blastocyst.



Mouse brain cleared using the CLARITY technique. Shown is the expression of Histone2B-GFP nuclear reporter in hypothalamic BSX neurons. The whole intact tissue imaging technique allows an accurate representation of the 3D morphology of BSX neurons in the mouse hypothalamus.



With optogenetics it is possible to stimulate or inhibit the BSX neuronal network in its entirety or only in subpopulations. Subsequently, we can determine the impact on neuronal excitablity in response to dietary stressors such as high fat and carbohydrate-rich diets.

Energy homeostasis and eating behavior

The ultimate goal for any living organism is to maintain energy homeostasis in its ability to survive. We are particularly interested in the neuronal circuits of the central nervous system (CNS) that are involved in the (dys) regulation of energy homeostasis. With the brain-specific homeobox protein BSX we identified an essential player marking a neuronal network within the hypothalamus that orchestrates eating behavior, sleep and higher cognitive functions.

It was generally believed that increased body weight always goes hand in hand with reduced physical activity. In other words, reducing body weight would lead to increased physical activity. However, we could show in ob/ob mice, which are deficient for leptin, that locomotor activity levels were not restored when body weight was reduced upon deletion of the BSX gene. Thus, our findings demonstrated for the first time that physical activity is at least in part genetically determined.

For us, the most fundamental question right now is to elucidate the molecular mechanisms that allow the BSX hypothalamic network to coordinate the dialogue between cognitive and metabolic pathways. We are in the process to characterize in detail the contributions of the different BSX neuronal subpopulations throughout the hypothalamus that contribute to this cross talk.

Experimentally, our studies will rely on the generation of novel mouse models with conditional inactivation of BSX itself or its identified targets in subpopulations of the BSX neuronal network. Subsequently, we will analyze the consequences of their altered expression with respect to feeding and sleep behavior.

Taking advantage of two state-of-the-art methods, optogenetics and CLARITY initially developed by the Deisseroth lab in Stanford, California, we will further unravel the function of the BSX neuronal network in the brain by correlating brain architecture to behaviors such as eating, movement and cognition.

Optogenetics is a technique that combines optics and genetics in order to control and measure the activity of certain neurons in animal tissue. It involves inserting light-sensitive proteins from algae into neurons, allowing researchers to switch neuronal activity on and off with light. This will allow us to stimulate or inhibit the BSX neuronal network in its entirety or only in subpopulations in order to understand its role in the regulation of behavioral and metabolic pathways. In this respect, we will also challenge the BSX-deficient neuronal network by dietary stressors including high fat and carbohydrate-rich diets to explore their impact on neuronal excitability.

In order to deconstruct the BSX hypothalamic network we will also employ **CLARITY** (**C**lear, **L**ipid-exchanged, **A**natomically **R**igid, **I**maging compatible, **T**issue h**Y**drogel), a technique that turns the brain transparent using the detergent SDS, which strips away lipids that normally block the passage of light. Here, the brain is first infused with acrylamide, which binds proteins, nucleic acids and other biomolecules. When the acrylamide is heated, it polymerizes and forms a tissue-wide mesh that secures the molecules. By using this technique it is possible to visualize and trace, quantify and map e.g. the axonal projections of the histaminergic neurons in an intact system in whole fluorochrome-expressing brains.

Selected Publications

Coldren CD, Lai Z, Shragg P, Rossi E, Glidewell SC, Zuffardi O, Mattina T, Ivy DD, Curfs LM, Mattson SN, Riley EP, Treier M, Grossfeld PD (2009). Chromosomal microarray mapping suggests a role for BSX and Neurogranin in neurocognitive and behavioral defects in the 11q terminal deletion disorder (Jacobsen syndrome). Neurogenetics. 2009 Apr;10(2):89-95

Uhlenhaut N, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, Treier AC, Klugmann C, Klasen C, Holter N, Riethmacher D, Schütz G, Cooney A, Lovell-Badge R & Treier M (2009). Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. Cell 139:1130-1142.

Attanasio M, Uhlenhaut NH, Sousa V, O'Toole JF, Otto E, Anlag K, Klugmann C, Treier AC, Helou J, Sayer JA, Seelow D, Nürnberg G, Becker C, Chudley AE, Nürnberg P, Hildebrandt F & Treier M (2007) Loss of GLIS2 causes nephronophthisis in humans and mice by increased apoptosis and fibrosis. Nature Genetics 39:1018-1024.

Sakkou M, Wiedmer P, Anlag K, Hamm A, Seuntjens E, Ettwiller L,Tschop M & Treier M (2007). A Role for Brain-Specific Homeobox Factor Bsx in the Control of Hyperphagia and Locomotory Behavior. Cell Metabolism 5:450-463

Elling U, Klasen C, Eisenberger T, Anlag K & Treier M (2006). Murine inner cell mass derived lineages depend on Sall4 function. PNAS 103(44), 16319-24.

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Cancer Research

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Cancer Research

Claus Scheidereit

Almost half a million cancer cases are diagnosed in Germany each year, making advancements in diagnosis, treatment and prevention of cancer a major challenge in health research. Our mission is to understand the principles of cancer development and progression and to provide the basis for improved diagnosis and treatment. One aim is to discover and characterize genes and cellular components that are important in the development and treatment of cancer and that are promising as novel targets for therapies. Another aim is to understand and therapeutically utilize interactions of the immune system with tumors.

The MDC Cancer Research Program covers the three topics, (i) signaling pathways, cell biology and cancer, (ii) structural and functional genomics and (iii) tumor immunology and combines a spectrum of interdisciplinary basic research laboratories and clinically oriented groups. A broad spectrum of experimental expertise is covered, ranging from cell biology, biochemistry, structural, developmental and stem cell biology to immunology, experimental tumor models and clinical oncology. Cancer studies are conducted in close collaboration with clinically oriented groups at the Charité - Medical Faculty Berlin.

In the reporting period, the international German-Israeli Research School "Frontiers in Cell Signaling and Gene Regulation, SignGene" (spokesman: **Claus Scheidereit**), funded by the Helmholtz Association, was established as a joint program of the MDC, the Hebrew University of Jerusalem (HUJI), the Technion - Israel Institute of Technology (TEC) in Haifa, the Humboldt University and the Charité. **Oliver Daumke** has been awarded one of the prestigious European Research Council (ERC) grants. He plans to investigate the internal structure and dynamics of mitochondria, to gain a precise molecular understanding of mitochondria-based processes relevant for diseases, such as cancer.

Selected scientific highlights

Chronic myeloid leukemia (CML) often results from chromosomal translocations, generating the oncogenic tyrosine kinase BCR-ABL. The impact of other disease promoting pathways is poorly understood. The group of **Achim Leutz** reported that the Wnt signal transducer β-catenin acts as an amplifier of a CML-specific gene signature. Activation of β-cateninin combination with the deletion of the interferon inducible transcription factor Irf8 results in highly aggressive leukemic stem cells and causes the progression of CML into a fatal blast crisis, implying Irf8 and β-catenin as future targets in combinatorial therapies (Scheller et al., J. Exp. Med., 2013).

Estrogen-receptor (ER)-negative basal breast cancers represent an aggressive subtype with poor prognosis. The group of **Walter Birchmeier** modeled cancer formation by combining activating mutations of Wnt/β-catenin and HGF/Met in mouse mammary gland epithelial cells and determined a Wnt-Met dependent gene signature, which predicts poor survival of human patients. The chemokine system CXCL12/CXCR4 was identified as a crucial driver of Wnt-Met tumors. Thus, targeting CXCR4, Wnt and Met might provide an efficient strategy for breast cancer treatment (Holland et al., Cell Rep, 2013).

To model Burkitt lymphoma (BL), **Klaus Rajewsky** and colleagues used targeted conditional mutagenesis, combining oncogenic c-MYC overexpression and constitutive PI3K signaling in germinal center B cells. The resulting mice reproduce the human disease in terms of histological appearance, gene expression signature, and

"tertiary" mutations. Overexpression of c-MYC and constitutive PI3K signaling turned out to be critical for the progression of the human disease. The BL model allows the identification of new drug targets and the elucidation of disease progression, such as the dissemination into the central nervous system (Sander et al., Cancer Cell, 2012).

T cell surveillance protects against virus-associated tumor development, but occasionally fails, particularly in case of hepatitis B/C virus-associated hepatocellular carcinoma (HCC). To explore this phenomenon, the group of **Thomas Blankenstein** infected mouse hepatocytes with oncogenic SV40 large T antigen (TAg) expressing adenoviruses. Infection induced cytotoxic T lymphocytes (CTLs), leading to clearance of the cells. However, a few virus-infected cells "sneaked through" and progressed to HCC. T cells can only poorly infiltrate HCC and the few infiltrating T cells are locally inhibited in their function. The established model may help to better understand human HCC (Willimsky et al., J. Clin. Invest., 2013).

To monitor and to combat viral infections and cancer, the immune system must be armed with efficient effector mechanisms, such as the cytokine interferon (IFN)-γ, released by T cells and NK cells. **Uta Höpken**, **Armin Rehm** and colleagues showed that the sorting receptor Sortilin controls exocytic trafficking of IFN-γ and of granzyme A. Sortilin-deficient mice revealed decreased release of interferon-γ, resulting in impaired immune responses upon infection and reduced autoimmune responses in inflammatory bowel disease (Herda et al., Immunity, 2012).

The control of lineage commitment of haematopoietic stem (HSCs) and progenitor cells is largely unknown. The Inserm/CNRS- and MDC-associated group of **Michael Sieweke** found that macrophage colony-stimulating factor (M-CSF) promotes HSC differentiation into myeloid cells. M-CSF produced upon infection or inflammation induces the expression of transcription factor PU.1 in HSCs, to promote differentiation into the myeloid cell lineage. Thus, inflammatory and stress conditions directly influence hematopoiesis at the level of HSCs (Mossadegh-Keller et al., Nature, 2013).

The ubiquitin proteasome system (UPS) maintains the integrity of cellular processes by controlling protein degradation, which is important for many cellular functions. Degradation is triggered by the protein ubiqutination and subsequent recognition of ubiquitin-binding domain (UBD) containing proteins. The group of **Thomas Sommer** explored the mechanistic details of ubiquitin binding to Cue1, a crucial regulator of the Endoplasmic Reticulum (ER)-associated protein degradation (ERAD) pathway (Bagola et al., Mol Cell, 2013).

Activated oncogenes or chemotherapy can lead to cellular senescence, an irreversible exit from the cell cycle. In this state, tumor cells do not proliferate but induce inflammatory reactions, which can promote tumor formation. The group of **Clemens Schmitt** observed that therapy-induced senescent cancer cells display changes in energy metabolism (increased glucose and oxygen consumption and ATP production), most likely due to massive proteotoxic stress, which is a consequence of the senescence-associated secretory phenotype (SASP). Both, inhibition of glucose production or blocking of autophagy caused cell death of the senescent tumor cells, implying a novel two-step therapy approach, which combines chemotherapy and metabolic therapy (Dörr et al., Nature, 2013).



Photo: David Ausserhofer/MDC

Miguel Andrade

Computational Biology and Data Mining

Our group develops and applies computational methods for the study of gene and protein function with emphasis on molecules related to human disease. Recently, our main fields of research have been the study of protein interaction networks, gene expression, and data and text mining of the biomedical literature.

Study of protein interaction networks

Martin Schaefer, Jean-Fred Fontaine

We created HIPPIE, a database that integrates protein-protein interaction (PPI) data from several databases scored according to attached experimental information. We recently showed that measured interactions where the interacting proteins are expressed in the same tissue, or cellular location, and involved in similar processes are more reliable (with Yoshihiro Kawaoka and Hiroaki Kitano). These properties are useful to focus on relevant PPI subnetworks; we implemented mechanisms to do this in HIPPIE and illustrated how to apply this to the study of biological cases.

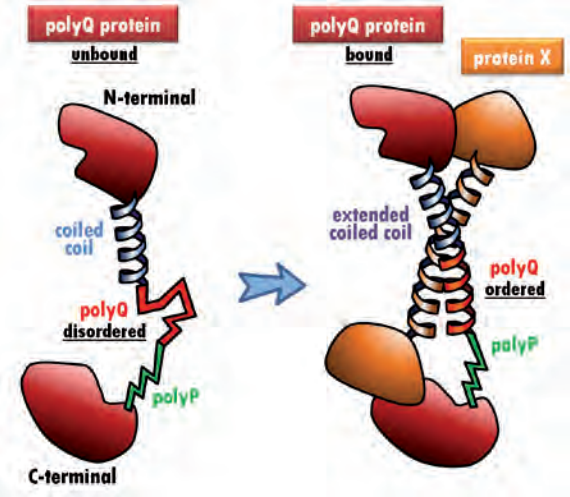
Expanded polyglutamine (polyQ) stretches are observed in the proteins of patients with different neurodegenerative diseases and are thought to trigger disease. We integrated genomic, phylogenetic, protein interaction network and functional information to add evidence that polyQ tracts modulate protein interactions (with Erich Wanker) (see Figure). PolyQ expansion results in gain of abnormal interactions, leading to pathological effects like protein aggregation. Furthermore, we characterized proteins that interact with ataxin-1, which has a polyQ tract whose abnormal expansion results in disease. Interactors that enhance the toxicity of mutant ataxin-1 are enriched in coiled-coil regions. We propose that while interactions of coiled-coil proteins with polyQ regions might increase aggregation, blocking this interaction, or interacting with other regions, might reduce aggregates and neurodegeneration.

Protein sequence analysis

David Fournier, Arvind Mer, Nancy Mah

We have updated a neural network method (ARD2) that identifies repeats like HEAT and Armadillo, which form similar structures composed of alpha-helices. We evaluated the phylogenetic distribution of these repeats, pointing to multiple likely events of independent emergence of these repeats in distant taxa and to their increased frequency in organisms of high cellular complexity such as eukarya in general, and cyanobacteria and planctomycetes within prokarya.

In general, proteins perform their functions in concrete sub-cellular locations (e.g., cytoplasmic, nuclear, extra-cellular). It was hypothesized that proteins adapt to their physicochemical environment by mutations on the residues exposed to the media not closely implicated in function and that therefore the composition of the exposed residues of a protein can be used for the prediction of protein sub-cellular location. We studied the predictive power of amino acid composition at variable ranges from buried to exposed and found that both buried and exposed amino acids carry complementary information about subcellular location. An optimized two step predictor trained with vectors of amino acid composition in different ranges of exposure and using a Support Vector Machine followed by a Neural Network (NYCE), reaches an accuracy of 62% when predicting nuclear, nucleocytoplasmic, cytoplasmic or extracellular location of eukaryotic proteins.



How polyQ modulates protein-protein interactions. *Left: unbound state of a protein containing a polyQ region (red) in disordered state, N-terminally preceded by a coiled-coil region (blue), and C-terminally followed by a disordered polyP region (green). Right: upon interaction of the polyQ protein with a protein X (brown) through a coiled-coil interaction, the polyQ region adopts a coil conformation extending the preceding (blue) coiled coil, therefore increasing the strength of the interaction. The polyP region cannot adopt a coiled-coil conformation effectively providing a cap to the interaction-dependent conformational change induced by the polyQ.*

Transcript regulation and prediction

Enrique Muro, Nancy Mah, Marie Gebhardt

Upstream open reading frames (uORFs) are (generally) short open reading frames that appear in the 5'UTRs of many transcripts. uORFs may interfere with the protein expression of a transcript but activatory effects have also been described. In collaboration with Achim Leutz, we are studying experimentally and computationally their properties and we developed uORFdb, a database about uORFs in eukaryotic organisms.

We are also developing computational methods to predict ncRNAs and to evaluate experimental results identifying ncRNAs, including transcripts arising from pseudogenes and the characterization of miRNA function based on the overlap of their targets to those of particular transcriptional repressors as derived from ChIP-seq experiments.

Analysis of gene expression

Nancy Mah, Jean-Fred Fontaine, Marie Gebhardt, Sander Bollen

Recently we have collaborated with several groups on the interpretation of profiles of gene expression: to demonstrate that higher expression of GPx3 correlates with higher proliferation of Leukemia hematopoietic Stem Cells (with Guy Sauvageau), to show that knock-down of transcription factor Irf8 transforms dendritic cell progenitors into neutrophils indicating that the DC progenitors have a default commitment (with Frank Rosenbauer), to define a signature of colorectal cancer recurrence based on the lower expression of immune-system related genes (with Wolfgang Kemmner), and with Francesca Spagnoli in the study of the molecular mechanisms involved in fate decision for progenitors that originate liver and pancreas.

We also develop methods for the study of DNA microarray data, for example to detect chromosomal regions with gross deletions or duplications by their abnormally low or high general gene expression. We have implemented a method (CAFE) as an R package to do such analysis and visualize the results.

Data and text mining

Jean-Fred Fontaine

We develop web tools to assist researchers exploring the biomedical literature to understand the molecular basis of disease. Recently, we developed the Alkemio web server to rank thousands of chemicals for their relevance to any topic according to their associated bibliography (in PubMed). We are also working on methods to mine the biomedical literature for relevant manuscripts. In this respect, we recently examined how the references cited by a manuscript improve document retrieval, and also how to associate experts to particular topics using the lists of authors of manuscripts in PubMed. We provide the latter analysis as a tool to find peer reviewers appropriate for a given manuscript (peer2ref).

Selected Publications

Schaefer, M.H., E.E. Wanker and M.A. Andrade-Navarro. 2012. Evolution and function of CAG/polyglutamine repeats in protein-protein interaction. Nucleic Acids Research. 40, 4273-4287.

Gebhardt, M.L., S. Reuter, R. Mrowka, and M.A. Andrade-Navarro. 2014. Similarity in targets with REST points to neural and glioblastoma related miRNAs. Nucleic Acids Research. In press.

Fournier, D., G.A. Palidwor, S. Shcherbinin, A. Szengel, M.H. Schaefer, C. Perez-Iratxeta and M.A. Andrade-Navarro. 2013. Functional and genomic analyses of alpha-solenoid proteins. PLoS ONE. 8, e79894.

Mer, A.S. and M.A. Andrade-Navarro. 2013. A novel approach for protein subcellular location prediction using amino acid exposure. BMC Bioinformatics. 14, 342.

Bollen, S., M. Leddin, M.A. Andrade-Navarro and N. Mah. 2014. CAFE: an R package for the detection of gross chromosomal abnormalities from gene expression microarray data. Bioinformatics. In press.

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Photo: David Ausserhofer/MDC

Walter Birchmeier

Signal Transduction in Development and Cancer

Our research is primarily focused on two signaling systems: Wnt/beta-catenin and receptor tyrosine kinase signaling. Components of both systems are frequently mutated or deregulated in a variety of developmental disorders and cancers in humans.

In the previous years, the laboratory has studied adhesion and signaling of E-cadherin/Wnt/beta-catenin by biochemical means. We have shown that beta-catenin binds to the transcription factors Lef1/Tcf, and that this translocates beta-catenin to the nucleus. Beta-catenin is recruited to its degradation complex containing Axin2/Conductin and APC. We have also investigated the role of scatter factor/hepatocyte growth factor (SF/HGF) and its receptor, the Met tyrosine kinase, in the morphogenesis of epithelial cells. Signals of Met are transmitted by the cytoplasmic multi-adapter Gab1 and the tyrosine phosphatase Shp2. Conventional ablations of beta-catenin and Gab1 in mice result in gastrulation defects and embryonic organ failures, respectively.

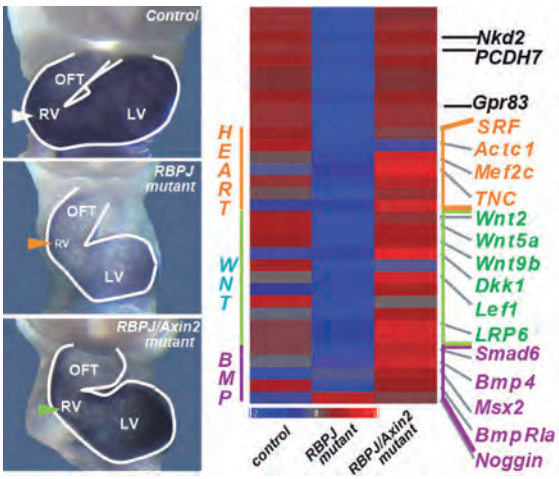
In recent years, we have examined the role of Wnt/beta-catenin and Met/Gab1/Shp2 by conditional mutagenesis in mice. Beta-catenin regulates precursor and stem cells in the nervous system, the hair and the heart; Gab1 and Shp2 control precursor cells in liver, limbs, and kidney. Met regulates wound healing in the skin. Activation of beta-catenin and HGF/Met in adult mouse tissues induces tumors and cancer stem cells.

In the present report period, we have published the following investigations:

Wnt/beta-catenin and Bmp signals control distinct sets of transcription factors in cardiac progenitor cells

A. Klaus, M. Müller, H. Schulz, Y. Saga, JF. Martin, W. Birchmeier

Progenitor cells of the first and second heart fields depend on cardiac-specific transcription factors for their differentiation. Using conditional mutagenesis of mouse embryos, we defined the hierarchy of signaling events that controls the expression of cardiac-specific transcription factors during differentiation of cardiac progenitors at embryonic day 9.0. Wnt/beta-catenin and Bmp act downstream of Notch/RBPJ at this developmental stage. Mutation of Axin2, the negative regulator of canonical Wnt signaling, enhances Wnt and Bmp4 signals and suffices to rescue the arrest of cardiac differentiation caused by loss of RBPJ. Using FACS enrichment of cardiac progenitors in RBPJ and RBPJ/Axin2 mutants, embryo cultures in the presence of the Bmp inhibitor Noggin, and by crossing a Bmp4 mutation into the RBPJ/Axin2 mutant background, we showed that Wnt and Bmp4 signaling activate specific and nonoverlapping cardiac-specific genes in the cardiac progenitors: Nkx2-5, Isl1 and Baf60c are controlled by Wnt/beta-catenin, and Gata4, SRF, and Mef2c are controlled by Bmp signaling. Our study contributes to the understanding of the regulatory hierarchies of cardiac progenitor differentiation and outflow tract development and has im-



Notch signaling induces the formation of the right ventricle of the heart (RV, on the left), given the reduction seen in the RBBJ mouse mutants (orange arrow). Forced Wnt/beta-catenin signaling substantially rescues this phenotype, as seen in the RBPJ/Axin2 double mutants (green arrow). A heatmap shows (on the right) that the expression of many genes is rescued in the Notch/Wnt double mutants, like heart (orange), Wnt (green) and BMP genes (magenta). From Klaus et al., PNAS 109, 1092-1096 (2012).

plications for understanding and modeling heart development (published in Proc. Natl. Acad Sci. USA, 109, 10921-10926, 2012).

Wnt/beta-catenin signalling induces MLL to create epigenetic changes in salivary gland tumors

P. Wend, L. Fang, Q. Zhu, JH. Schipper, C. Loddenkemper, F. Kosel, V. Brinkmann, K. Eckert, S. Hindersin, JD. Holland, S. Lehr, M. Kahn, U. Ziebold, W. Birchmeier

We show that activation of Wnt/beta-catenin and attenuation of Bmp signals, by combined gain- and loss-of-function mutations of beta-catenin and Bmpr1a, respectively, results in rapidly growing, aggressive squamous cell carcinomas (SCC) in the salivary glands of mice. Tumors contain transplantable and hyperproliferative tumor propagating cells, which can be enriched by fluorescence activated cell sorting. Single mutations stimulate stem cells, but tumors are not formed. We show that beta-catenin, CBP and Mll promote self-renewal and H3K4 tri-methylation in tumor propagating cells. Blocking beta-catenin-CBP interaction with the small molecule ICG-001 and small-interfering RNAs against beta-catenin, CBP or Mll abrogate hyperproliferation and H3K4 tri-methylation, and induce differentiation of cultured tumor propagating cells into acini-like structures. ICG-001 decreases H3K4me3 at promoters of stem cell-associated genes in vitro and reduces tumor growth in vivo. Remarkably, high Wnt/beta-catenin and low Bmp signalling also characterize human salivary gland SCC and head and neck SCC in general. Our work defines mechanisms by which beta-catenin signals remodel chromatin and control induction and maintenance of tumor propagating cells. Further, it supports new strategies for the therapy of solid tumors (published in EMBO J. 17, 1977-89, 2013).

Wnt/Rspondin/beta-catenin signals control axonal sorting and lineage progression in Schwann cell development

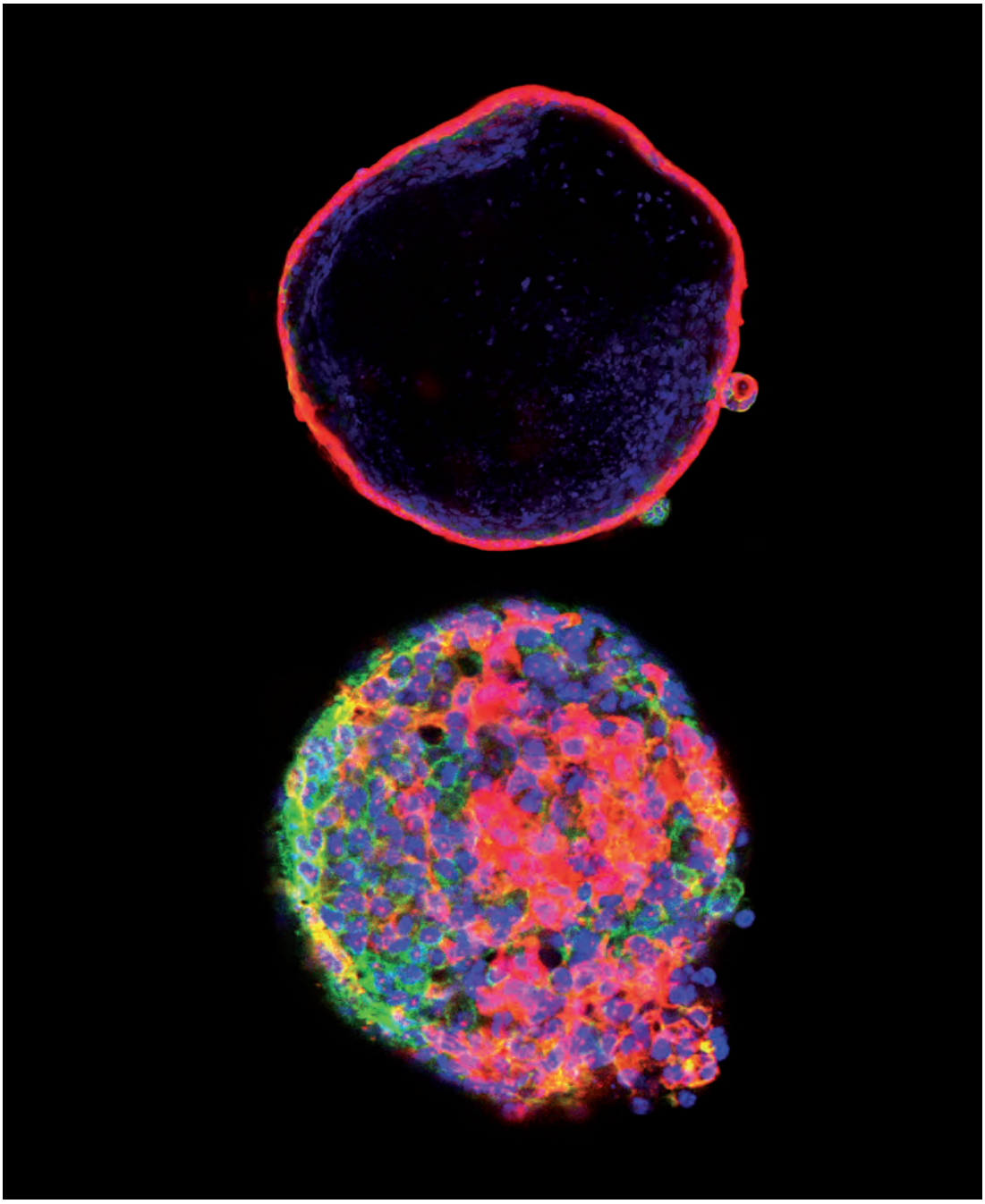
Tamara Grigoryan, Simone Stein, Jingjing Qi, Hagen Wende, Alistair N. Garratt, Klaus-Armin Nave, Carmen Birchmeier, and Walter Birchmeier

During late Schwann cell development, immature Schwann cells segregate large axons from bundles, a process called "axonal radial sorting". Here we demonstrate that canonical Wnt signals play a critical role in radial sorting and assign a role to Wnt and Rspondin ligands in this process. Mice carrying beta-catenin loss-of-function mutations show a delay in axonal sorting; conversely, gain-of-function mutations result in accelerated sorting. Sorting deficits are accompanied by abnormal process extension, differentiation, and aberrant cell cycle exit of the Schwann cells. Using primary cultured Schwann cells, we analyze the upstream effectors, Wnt and Rspondin ligands that initiate signaling, and downstream genetic programs that mediate the Wnt response. Our analysis contributes to a better understanding of the mechanisms of Schwann cell development and fate decisions (published in Proc. Natl. Acad. Sci. USA, 110, 18174-18179, 2013).

Combined Wnt/beta-Catenin, HGF/Met, and CXCL12/CXCR4 Signals Characterize Basal Breast Cancer and Predict Disease Outcome

Jane D. Holland, Balázs Győrffy, Regina Vogel, Klaus Eckert, Giovanni Valenti, Liang Fang, Philipp Lohneis, Sefer Elezkurtaj, Ulrike Ziebold, and Walter Birchmeier

Prognosis for patients with estrogen-receptor (ER)-negative basal breast cancer is poor, and chemotherapy is currently the



Cells derived from mammary gland tumors of Wnt-Met compound mutant mice grown as 3D organoids. It is shown that treatment with a combination of Wnt and Met inhibitors induce the appearance of normal-like mammary gland acini (upper): hollow structures composed of a polarized outer layer of basal epithelial cells (red staining for keratin 5) encapsulating a luminal cell layer (green for keratin 8). Untreated organoids produce filled, transformed structures (lower). From Holland et al., Cell Reports 5, 1214-1227 (2013).

best therapeutic option. We have generated a compound-mutant mouse model combining the activation of beta-catenin and HGF (Wnt-Met signaling), which produced rapidly growing basal mammary gland tumors. We identified the chemokine system CXCL12/CXCR4 as a crucial driver of Wnt-Met tumors, given that compound-mutant mice also deficient in the CXCR4 gene were tumor resistant. Wnt-Met activation rapidly expanded a population of cancer-propagating cells, in which the two signaling systems control different functions, selfrenewal and differentiation. Molecular therapy targeting Wnt, Met, and CXCR4 in mice significantly delayed tumor development. The expression of a Wnt-Met 322 gene signature was found to be predictive of poor survival of human patients with ER-negative breast cancers. Thus, targeting CXCR4 and its upstream activators, Wnt and Met, might provide an efficient strategy for breast cancer treatment (published in Cell Reports 5, 1214-1227, 2013).

Shp2/Mapk signaling controls goblet/paneth cell fate decisions in the intestine

Julian Heuberger, Frauke Kosel, Jingjing Qi, Katja Grossmann, Klaus Rajewsky and Walter Birchmeier

In the development of the mammalian intestine, Notch and Wnt/beta-catenin signals control stem cell maintenance and their differentiation into absorptive and secretory cells. Mechanisms that regulate differentiation of progenitors into the three secretory lineages, goblet, paneth or enteroendocrine cells, are not fully understood. Using conditional mutagenesis in mice, we observed that Shp2-mediated Mapk signaling determines the choice between paneth and goblet cell fates, and also affects Lgr5+ stem cells. Ablation of the tyrosine phosphatase Shp2 in the intestinal epithelium reduced Mapk signaling and led to a reduction of goblet cells while promoting paneth cell development. Conversely, conditional Mek1 activation rescued the Shp2 phenotype, promoted goblet cell and inhibited paneth cell generation. The Shp2 mutation also expanded Lgr5+ stem cell niches, which could be restricted by activated Mek1 signaling. Changes of Lgr5+ stem cell quantities were accompanied by alterations of paneth cells, indicating that Shp2/Mapk signaling might affect stem cell niches directly or via paneth cells. Remarkably, inhibition of Mapk signaling in intestinal organoids and cultured cells changed the relative abundance of Tcf4 isoforms and by this, promoted Wnt/beta-catenin activity. The data thus show that Shp2-mediated Mapk signaling controls the choice between goblet and paneth cell fates by regulating Wnt/beta-catenin activity (published in Proc. Natl. Acad. Sci. USA, 111, 3472-3477, 2014).

New projects of the laboratory are concerned with the function of the E3 ubiquitin ligase Hakai by conditional gene ablation and by proteomics approaches (Julian Heuberger, together with Matthias Selbach). In cooperation with the Leibniz Institute of Molecular Pharmacology in Berlin-Buch, we develop further small molecule inhibitors of beta-catenin/Tcf4 (Liang Fang, together with Edgar Specker and Jens von Kries) and Shp2 (Judith Holz and Marc Nazaré). In cooperation with the Department of Urology of the Charité Berlin, we characterise human kidney cancer stem cells (Annika Fendler, together with Klaus Jung and Jonas Busch). We also examine the function of Gab1 and Mapk signaling in hair cycle and stem cell quiescence (Özlem Akilli-Öztürk) and of Shp2 in cancer stem cells and senescence of PyMT mammary gland tumors (Linxiang Lan, together with EPO). We also study how Wnt/beta-catenin-target genes promote metastasis and migration of colorectal cancer cells (Jingjing Qi, in cooperation of EPO).

Selected Publications

Heuberger, J., Kosel, F., Qi, J., Grossmann, K.S., Rajewsky, K., and Birchmeier, W. 2014. Shp2/Mapk signaling controls goblet/paneth cell fate decisions in the intestine. Proc Natl Acad Sci USA, 111: 3472-3477.

Holland, J.D., Győrffy, B., Vogel, R., Eckert, K., Valenti, G., Fang, L., Lohneis, P., Elezkurtaj, S., Ziebold, U., and Birchmeier, W. 2013. Combined Wnt/beta-catenin, Met and CXCL12/CXCR4 signals characterize basal breast cancer and predicts disease outcome. Cell Reports 5: 1214-1227.

Grigoryan T, Stein S, Qi J, Wende H, Garratt AN, Nave KA, Birchmeier C, and Birchmeier W. 2013. Wnt/Rspondin/beta-catenin signals control axonal sorting and lineage progression in Schwann cell development. Proc Natl Acad Sci USA 110: 18174-18179.

Wend P, Fang L, Zhu Q, Schipper JH, Loddenkemper C, Kosel F, Brinkmann V, Eckert K, Hindersin S, Holland JD et al. 2013. Wnt/beta-catenin signalling induces MLL to create epigenetic changes in salivary gland tumours. EMBO J 32: 1977-1989.

Klaus A, Muller M, Schulz H, Saga Y, Martin JF, and Birchmeier W. 2012. Wnt/beta-catenin and Bmp signals control distinct sets of transcription factors in cardiac progenitor cells. Proc Natl Acad Sci USA 109: 10921-10926.

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Molecular Immunology and Gene Therapy

We focus on three areas in cancer immunology:
1. Developing novel cancer models that better reflect human disease.
2. Employing the mechanisms of rejection of established tumors by adoptively transferred T cells with specific emphasis of the tumor stroma as target.
3. Employing T cell therapy of cancer in the clinic. In the recent years we have developed a series of novel transgenic mouse models that allowed to analyze spontaneous anti-tumor responses, to track tumor or T cells by non-invasive bioluminescence imaging and to modulate oncogene expression in vivo. In addition transgenic mice are used that allow the isolation of TCRs to redirect T cells towards superior antitumor activity.

Virus-induced hepatocellular carcinomas cause antigen-specific local tolerance.

T cell surveillance is often effective against virus-associated tumors because of their high immunogenicity. It is not clear why surveillance occasionally fails, particularly against hepatitis B virus- or hepatitis C virus-associated hepatocellular carcinoma (HCC). We established a transgenic murine model of virus-induced HCC by hepatocyte-specific adenovirus-induced activation of the oncogenic SV40 large T antigen (Tag). Adenovirus infection induced cytotoxic T lymphocytes (CTLs) targeted against the virus and Tag, leading to clearance of the infected cells. Despite the presence of functional, antigen-specific T cells, a few virus-infected cells escaped immune clearance and progressed to HCC. These cells expressed Tag at levels similar to HCC isolated from neonatal Tag-tolerant mice, suggesting that CTL not select for cells with low immunogenicity. Virus-infected mice revealed significantly greater T cell infiltration in early-stage HCC compared with that in late-stage HCC, demonstrating progressive local immune suppression through inefficient T cell infiltration. Programmed cell death protein-1 (PD-1) and its ligand PD-L1 were expressed in all Tag-specific $CD8^+$ T cells and HCC, respectively, which contributed to local tumor-antigen-specific tolerance. Thus, we have developed a model of virus-induced HCC that may allow for a better understanding of human HCC.

Differently immunogenic cancers in mice induce immature myeloid cells that suppress CTL in vitro but not in vivo following transfer.

Tumors frequently induce immature myeloid cells (iMC), which suppress specific and unrelated CTL- responses and are termed myeloid-derived suppressor cells (MDSC). Mainly analyzed by in vitro assays in tumor transplantation models, little is known about their function in autochthonous tumor models in vivo. We analyzed iMC in 3 SV40 large T (Tag)-driven conditional autochthonous cancer models with different immune status: (1) Early Tag-specific CTL competence and rare stochastic Tag activation leading to sporadic cancer, which induces an aberrant immune response and CTL tolerance; (2) Cre/LoxP recombinase-mediated hepatocellular carcinoma (HCC) development in neonatal Tag-tolerant mice; and (3) Tag-activation through Cre recombinase-encoding viruses in the liver and HCC development with systemic anti-Tag CTL immunity. In the first but not two latter models, tumors induced CTL hyporesponsiveness to tumor-unrelated antigens. Regardless of the model, tumors produced interleukin-6 and vascular endothelial growth factor but not granulocyte macrophage–colony-stimulating factor (GM-CSF) and induced iMC (CD11b(+) Gr-1(int)) that suppressed CTL responses in vitro. None of the iMC from the different tumor models suppressed CTL responses in adoptive cell transfer experiments unless GM-CSF was provided in vivo. Together, iMC expand independent of the type of antitumor response and are not immunosuppressive in a cell-autonomous fashion.

Fas expression by tumor stroma is required for cancer eradication.

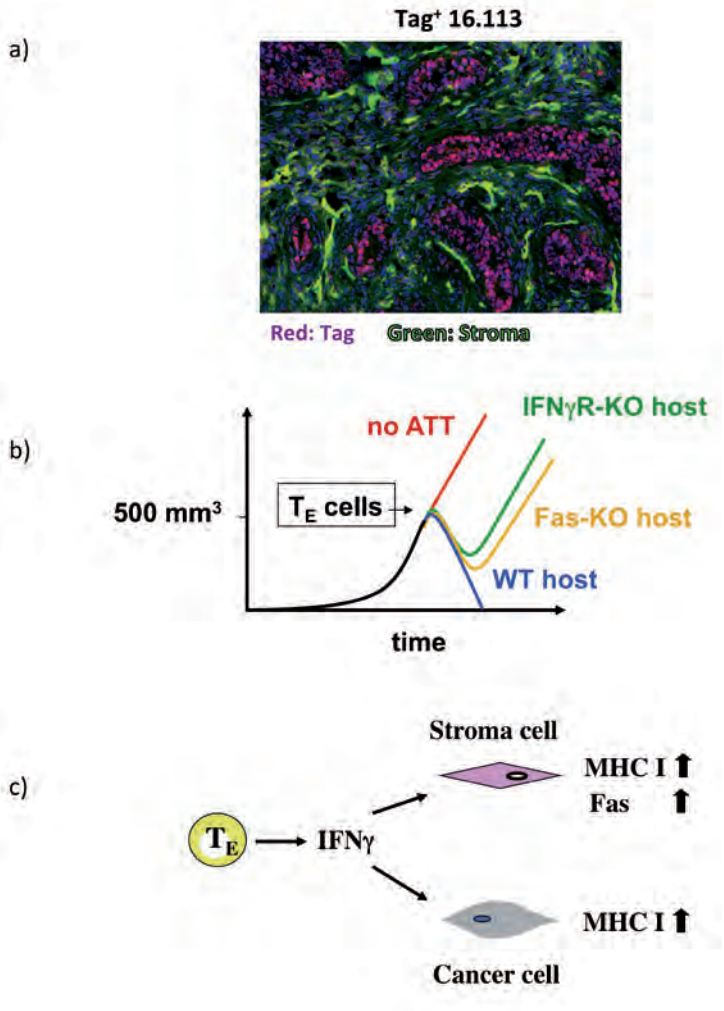
The contribution of molecules such as perforin, IFN-γ (IFNγ), and particularly Fas ligand (FasL) by transferred $CD8^+$ effector T (T_E) cells to rejection of large, established tumors is incompletely understood. Efficient attack against large tumors carrying a surrogate tumor antigen (mimicking a "passenger" mutation) by T_E cells requires action of IFNγ on tumor stroma cells to avoid selection of antigen-loss variants. Because "cancer-driving" antigens (CDAs) are rarely counterselected, IFNγ may be expected to be dispensable in elimination of cancers by targeting a CDA. Here, initial regression of large, established tumors required neither IFNγ, FasL, nor perforin by transferred $CD8^+$ T_E cells targeting SV 40 large T as CDA. However, cytotoxic T_E cells lacking IFNγ or FasL could not prevent relapse despite retention of the rejection antigen by the cancer cells. Complete tumor rejection required IFNγ-regulated Fas by the tumor stroma. Therefore, T_E cells lacking IFNγ or FasL cannot prevent progression of antigenic cancer because the tumor stroma escapes destruction if its Fas expression is down-regulated.

Depot formation of doxycycline impairs Tet-regulated gene expression *in vivo*.

The tetracycline (Tet) system is widely used for regulation of gene expression in vitro and in vivo. We constructed C57BL/6 transgenic mice (rtTA-CM2) with strong and ubiquitous reverse transactivator (rtTA2(S)-M2) gene expression. rtTA-CM2 mice were crossed to Tet-responsive reporter mice (LC-1) conditionally expressing the firefly luciferase (FLuc) gene under control of a Tet-responsive element, which allowed sensitive quantification of the transactivator activity by bioluminescent imaging. Following doxycycline (dox) application, up to 10^5-fold increase in BL signal was measured. rtTA activity was inducible in most analyzed organs. After dox withdrawal the BL signal decreased significantly but did not disappear completely, most likely due to a dox depot formation in vivo. The residual dox was sufficient to partly down-regulate a Tet-off controlled oncogene in a tumor transplantation experiment, resulting in reduced tumor growth. rtTA-CM2 mice may be a useful tool to analyze the function of genes in various organs but also reveal that down-regulation of gene expression is not complete.

B-cells and IL-4 promote methylcholanthrene-induced carcinogenesis but there is no evidence for a role of T/NKT-cells and their effector molecules (Fas-ligand, TNF-α, perforin).

Mice deficient either in subtypes of immune cells, cytokines or lytic pathways have been subjected to chemical carcinogenesis by methylcholanthrene to evaluate whether these components of the immune system affect tumor development. Inbred mice of the same genotype but from different sources differed in tumor development in magnitude comparable to that previously attributed to differences in immunocompetence. This suggested that genetic drift between separate inbred colonies of mice and/or environmental factors (e.g., transport of the animals) influenced carcinogenesis. Therefore, littermates were used as control in subsequent experiments. Although deficiency of T cells, NKT cells, perforin, Fas-ligand, TNF-α-receptor failed to reveal significant differences in tumor development, the presence of B cells and IL-4 enhanced tumor development under similar experimental conditions.



IFNγ maintains MHC I and Fas expression on tumor stroma.
a) Large established 16.113 tumors express SV 40 large as target antigen (Tas) and contain abundant stroma.

b) Rag$^{-/-}$, Rag$^{-/-}$/IFNγ-receptor$^{-/-}$ and Rag$^{-/-}$/Fas$^{-/-}$ mice with large 16.113 tumors were treated with SV40 large T antigen-immune CD8^{+} T cells (TE) and tumor growth was followed overtime. Schematic scheme of data published in Listopad et al., 2013. Note that expression of IFNγ-Receptor and Fas on the tumor stroma is necessary to prevent tumor recurrence.

c) In the absence of IFNγ, the tumor stroma escapes by MHC I/Fas down-modulation, which allows immunogenic cancer to progress.

Generation of transgenic mice with megabase-sized human yeast artificial chromosomes by yeast spheroplast-embryonic stem cell fusion.

Introducing human genes into mice offers the opportunity to analyze their in vivo function or to obtain therapeutic molecules. For proper gene regulation, or in case of multigene families, megabase (Mb)-sized DNA fragments often have to be used. Yeast artificial chromosome (YAC)-mediated transgenesis is irreplaceable for this purpose, because alternative methods such as the use of bacterial artificial chromosomes (BACs) cannot introduce DNA fragments larger than 500 kb into the mouse germ line. However, YAC libraries often contain only partial gene loci. Time-consuming reconstruction of YACs, genetic instability and the difficulty in obtaining intact YAC DNA above a certain size impede the generation of humanized mice. Here we describe how to reconstruct YACs containing Mb-sized human DNA, such as the T cell receptor-α (TRA) gene locus, thus facilitating the introduction of large DNA fragments into the mouse germ line. Fusion of YAC-containing yeast and embryonic stem (ES) cells avoids the need for YAC DNA purification. These ES cells are then used to stably introduce the functional TRA gene locus into the mouse germ line. The protocol takes 1 year to complete, from reconstruction of the entire TRA gene locus from YACs containing partial but overlapping TRA regions to germline transmission of the YAC.

Selected Publications

Willimsky, G., Schmidt, K., Loddenkemper, C., Gellermann, J. and **Blankenstein, Th.** (2013). Virus-induced hepatocellular carcinomas cause antigen-specific local tolerance. **J. Clin. Invest.** 123: 1032-1043.

Listopad, J., Kammertoens, T., Anders, K., Silkenstedt, B., Willimsky, G., Schmidt, K., Kühl, A., Loddenkemper, C. and **Blankenstein, Th.** (2013). Fas expression by tumor stroma is required for cancer eradication. **Proc. Natl. Acad. Sci. USA** 110: 2276-2281.

Schmidt, K., Zilio, S., Schmollinger, J., Bronte, V., **Blankenstein, Th.** and Willimsky, G. (2013). Differently immunogenic cancers in mice induce immature myeloid cells that suppress CTL in vitro but not in vivo following transfer. **Blood** 121: 1740-1748.

Kammertoens, T. and **Blankenstein, Th.** (2013). It's the peptide-MHC affinity, stupid. **Cancer Cell** 23: 429-431.

Li, L.-P. and **Blankenstein, Th.** (2013). Generation of transgenic mice with megabase-sized human yeast artificial chromosomes by yeast spheroplast-embryonic stem cell fusion. **Nat. Protoc.** 8: 1567-1582.

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Clinical and Molecular Oncology

Anticancer drug therapies rely on the induction of cell cycle arrest or cell death through cellular stress programs. Deregulation of such stress pathways in cancer cells by genetic or epigenetic inactivation results in evasion of the tumor cells from cancer therapy, insufficient responses to treatment with selection of therapy-resistent subclones and, consequently, tumor relapse, metastasis coinciding with poor disease prognosis. The aim of the group is to identify genetic defects in cancer that result in such aggressive disease and resistance to clinical cancer therapy. Recent data from our group indicate that specific defects in cell signaling for apoptosis, autophagy or other forms of cell death such as mitotic catastrophe can be overcome by rational selection of targeted anticancer drugs. Further projects are aimed to gain insights into novel aspects of cell cycle and cell death regulation and their intricate interactions, e.g. in the context of tumor suppression by p14^{ARF} or targeted cancer therapies.

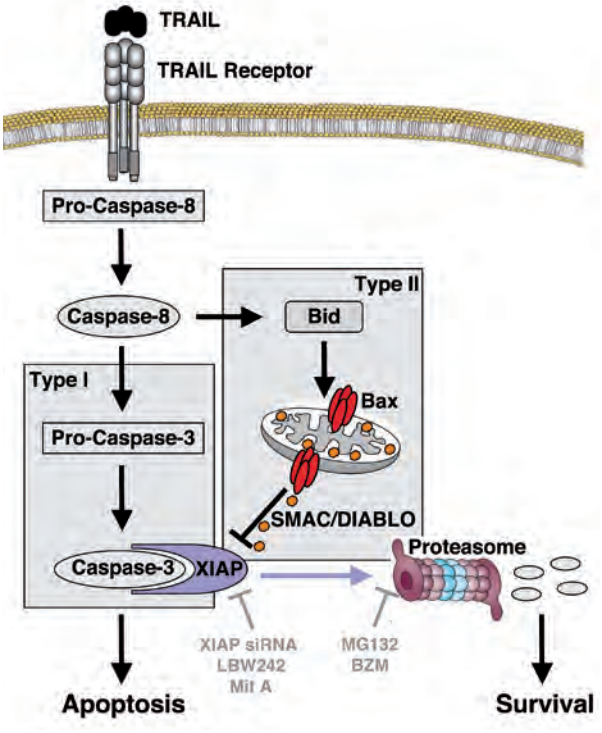
Understanding resistance to anticancer therapy

In cooperation with F. Essmann (Interfaculty Institute for Biochemistry, Univ. Tübingen), C. Belka (Clinic and Polyclinic for Radiation Therapy and Radiation Oncology, LMU München), and T. Blankenstein (MDC); supported by the German Consortium for Translational Cancer Research (DKTK)

Many anticancer therapies activate nuclear stress responses to induce cell cycle arrest and DNA repair. When repair fails, the same stress responses trigger cellular senescence or death by apoptosis or autophagy and demise of the affected cell. The molecular basis of these events has been studied extensively during recent years and comprehensive models are now established for large parts of these signaling events. Novel disease-related resistance mechanisms and therapeutic targets were identified by our group in leukemias and solid tumors. In this context, we recently described, e.g. selective loss of multiple cell death genes, including BH3-only proteins Nbk and Bim, in carcinoma of the kidney. This is a unifying feature of renal carcinoma and appears to be linked to the impressive clinical resistance of this tumor entity to anticancer therapy. Similar events were identified in acute leukemias. Links to epigenetic regulation and mTOR/PI3 kinase signaling were addressed.

Regulation of cell death by pro-apoptotic Bcl-2 family members

Apoptosis is mediated through at least three major pathways that are regulated by (1) the death receptors, (2) the mitochondria, and (3) the endoplasmic reticulum (ER). In most cells, these pathways are controlled by the Bcl-2 family of proteins that can be divided into anti-apoptotic (Bcl-2, Bcl-x_L, Mcl-1) and pro-apoptotic members. Although the overall amino acid sequence homology between the family members is relatively low, they contain highly conserved domains, referred to as Bcl-2 homology domains (BH1 to BH4) that are essential for homo- and hetero-complex formation as well as for their cell death inducing capacity. Structural and functional analyses revealed that the pro-apoptotic homologs can be subdivided into the Bax subfamily (Bax, Bak, Bok) and the growing BH3-only subfamily. BH3-only proteins link upstream signals from different cellular or functional compartments to the mitochon-



Therapeutic targeting of XIAP as a switch between Type I and Type II apoptosis signaling in therapy resistant malignant tumors

The death ligand TRAIL (TNF-related apoptosis inducing ligand) initiates TRAIL-receptor-mediated recruitment and activation of the death protease zymogen pro-caspase-8. In type I cells, this is sufficient to induce caspase-3 processing and execution of apoptosis. Most tumor cells are, however, of a type II and rely upon a mitochondrial, Bcl-2 family regulated cell death pathway to achieve sufficient activation of executioner caspases. Mitochondrial release of SMAC/DIABLO, the second mitochondrial activator of caspases, into the cytosol thereby plays a key role in caspase activation by displacing the E3-Ubiquitin-Ligase XIAP from binding to activated caspase-3 thereby impeding proteasomal degradation. Inhibition of XIAP by small molecule SMAC mimetics, e.g. LBW242, or the SP1 inhibitor Mit A or XIAP siRNA facilitates caspase-3 activation, converts cells from a type II to a type I. This may circumvent the frequently found central apoptosis defects in malignant tumors that are caused by up-regulation of anti-apoptotic Bcl-2 members (e.g. Bcl-2, Bcl-xL, Mcl-1), loss of pro-apoptotic Bax and/or Bak, or BH3-only proteins. Proteasome inhibitors such as bortezomib (BZM) and MG132 restore caspase-3 activity by interfering with proteasomal degradation. Mit A: Mithramycin A.

drial apoptosis pathway. BH3-only proteins Puma, Noxa, Hrk, and Nbk (Bik) are induced by p53 and mediate cell death originating from the nucleus, e.g. upon DNA damage. Nbk localizes to the ER and activates the pro-apoptotic multidomain protein Bax (but not the homologous Bak) indirectly, through a ER-initiated death pathway that has been recently elucidated by our group. Bid links the death receptor pathway to the mitochondrial death machinery where it triggers activation of Bax and Bak following limited proteolysis by caspase-8/-10 to tBid.

Aims of our work are (1) to gain structural and functional insights into how Bcl-2 subfamilies promote or inhibit cell death signals and how these properties may be utilized for development of cell death-promoting cancer therapies, (2) to address interactions with oncogene sigalling and p53 dependent and independent sigalling by the $p14^{ARF}$ tumor suppressor and (3) to understand pathway interactions with cell death signalling as resistance mechanisms to targeted cancer therapy, e.g. in mTOR/PI3K survival signalling.

We recently showed how to resensitize tumor cells for drug- induced cell death in Bax-deficient carcinomas by targeting the anti-apoptotic Bcl-2 homolog Mcl-1. We also found that such central defects in cell death resistance can be overcome even more efficiently by a new class of apoptosis targeting drugs that mimic the function of the endogenous cell death activator SMAC that interferes with protection from cell death by the Inhibitor of Apoptosis Proteins (IAP) with XIAP playing a dominant role in TRAIL resistance. These studies also showed that interference with the E3-ubiquitin ligase function of XIAP appears to be an important mechanism of proteasome targeting drugs such as Bortezomib.

Selected Publications

Gillissen B, Richter A, Richter A, Overkamp T, Essmann F, Hemmati PG, Preissner R, Belka C, Daniel PT. Targeted therapy of the XIAP/proteasome pathway overcomes TRAIL-resistance in carcinoma by switching apoptosis signaling to a Bax/Bak-independent 'type I' mode. Cell Death Dis. 2013, 23:e643

Zaltsman Y, Shachnai L, Yivgi-Ohana N, Schwarz M, Maryanovich M, Houtkooper RH, Vaz FM, De Leonardis F, Fiermonte G, Palmieri F, Gillissen B, Daniel PT, Jimenez E, Walsh S, Koehler CM, Roy SS, Walter L, Hajnóczky G, Gross A. MTCH2/MIMP is a major facilitator of tBID recruitment to mitochondria. Nat Cell Biol. 2010, 12:553-62.

Anders K, Buschow C, Herrmann A, Milojkovic A, Loddenkemper C, Kammertoens T, Daniel P, Yu H, Charo J, Blankenstein T. Monospecific CD8+ effector T cells reject large genetically unstable tumors, while oncogene inactivation selects escape variants. Cancer Cell, 2011, 20: 755-67.

Gillissen B, Wendt J, Richter A, Richter A, Müer A, Overkamp T, Gebhardt N, Preissner R, Belka C, Dörken B, Daniel PT. Endogenous Bak inhibitors Mcl-1 and Bcl-xL: differential impact on TRAIL resistance in Bax-deficient carcinoma. J Cell Biol. 2010, 188:851-62.

Gillissen B, Essmann F, Hemmati P, Richter A, Richter A, Öztop I, Chinnadurai G, Dörken B and Daniel PT. Mcl-1 mediates the Bax dependency of Nbk/Bik-induced apoptosis. J Cell Biol 2007, 179: 701-15.

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Photo: David Ausserhofer/MDC

Oliver Daumke

Video:
How does the architecture of a protein help to block infections?

Structure and Function of Membrane-associated GTPases

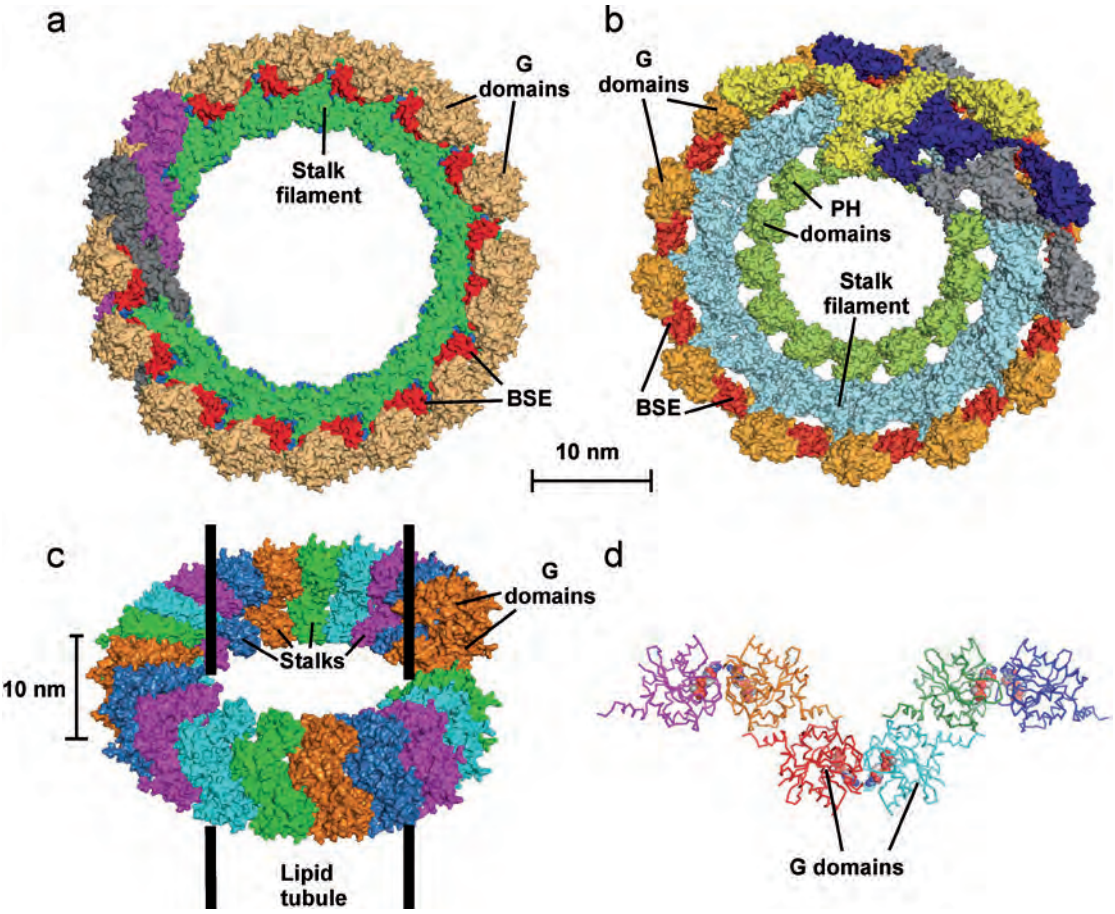
GTPases of the dynamin and septin superfamilies are molecular machines that assemble at the surface of cellular membranes. Some of these proteins act as dynamic scaffolds that remodel the underlying membrane in a GTPase-dependent fashion; others orchestrate the recruitment of interaction partner in a temporally and spatially defined manner. We are interested to understand principles of assembly, function and regulation of these GTPases. To this end, structural studies are combined with biochemical and cell-based approaches.

Structure and antiviral function of MxA GTPases

The interferon-induced Myxovirus-resistance (Mx) GTPases are central players of innate immunity. They mediate resistance against a wide range of pathogens, including influenza viruses and many other negative-strand viruses. As typical members of the dynamin superfamily, they can oligomerize in ring-like structures around tubular membranes.

Using X-ray crystallography, we found that the virus binding domain, the stalk, of human MxA has a four-helix bundle fold. In the crystals, the stalks assembled via three interfaces in a zigzag fashion. We showed that all three interfaces are required for the assembly of MxA at membrane surfaces and for its antiviral activity. Based on these results, we suggested a model how MxA assembles into ring-like oligomers and how these rings mediate the antiviral activity (Fig. 1a).

We also determined the structure of the full-length MxA molecule. We identified the bundle signalling element (BSE), a three-helix bundle, which bridges GTPase (G) domain and stalk. Based on functional experiments, we suggested a molecular pathway how nucleotide-driven rearrangements in the MxA oligomer are transmitted from the GTPase domain to the stalk.

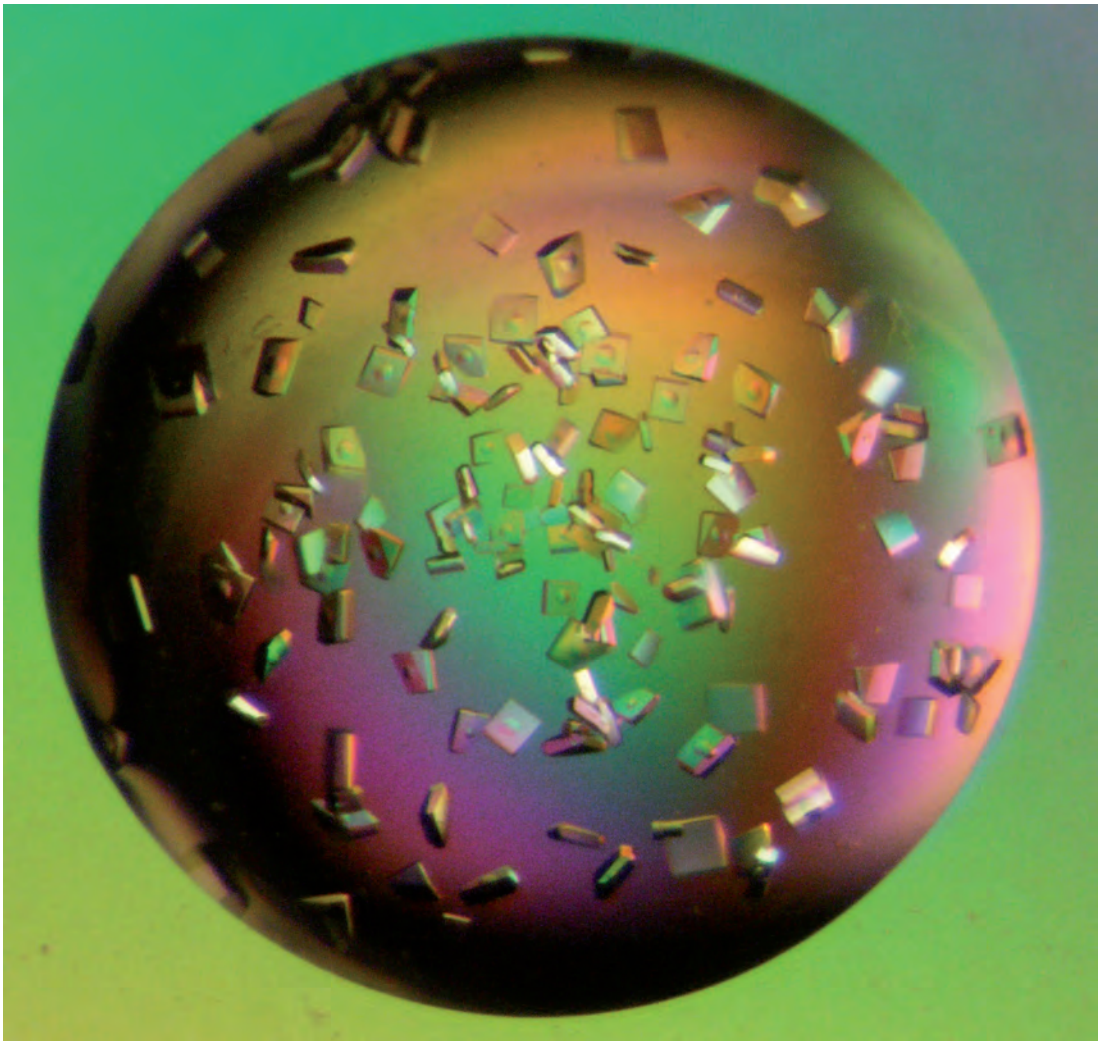


Oligomerization of dynamin and septin-like GTPases.
a) Structural model of ring-like oligomers of the MxA GTPase. We suggested that these rings assemble around ribonucleoprotein complexes of negative-strand RNA viruses, thereby interfering with viral replication. b) Model of helical dynamin oligomers. Similarly to MxA, assembly proceeds via three conserved interfaces in the stalks. The dynamin helix oligomerizes around the neck of clathrin-coated vesicles leading to vesicle scission. c) Oligomeric ring model of the EHD2 ATPase. Oligomerization is solely mediated via the GTPase domains, but not the helical domains. This assembly mode suggests an alternative mechanism of EHD2 in membrane remodelling. d) Linear GTP-dependent oligomer of GIMAP2. GIMAP2 assembles via two interfaces in the GTPase domain into a linear scaffold. An amphipathic C-terminal a-helix might mediate assembly of interaction partners.

Structural insights into dynamin-mediated endocytosis

Dynamin is the founding member of the dynamin superfamily. The multi-domain protein oligomerizes around the neck of clathrin-coated vesicles and induces membrane scission in a GTPase-dependent fashion. We are interested to understand the molecular mechanisms how dynamin mediates membrane scission.

Based on our MxA work, we determined the X-ray structure of nearly full-length dynamin. Dynamin shows a four-domain architecture comprising a GTPase domain, a BSE, a stalk and a lipid-binding pleckstrin homology (PH) domain. Interestingly, we found that the interaction site between the stalk and the PH domain is often mutated in patients suffering from centronuclear myopathy, a congenital disease leading to progressive muscle weakness. Supported by biochemical and cell-based functional experiments, we proposed a molecular model for helical dynamin oligomers (Fig. 1b). Furthermore, we suggested how the interplay between the dynamin domains contributes to the mechano-chemical coupling. Finally, we introduced a dynamic model how GTP binding and hydrolysis induces scission of the vesicle neck.



Crystals of GIMAP2, observed under polarized light, with a typical size of 0.2 x 0.1 x 0.05 mm3. In these crystals, the GIMAP2 molecules are arranged in a 3-dimensional lattice. Using X-ray diffraction, 3D information on the structure of GIMAP2 was obtained.

Dynamin-related GTPases in mitochondrial remodelling

A new focus of our group has been the structural and functional characterization of mitochondrial membrane remodelling events. Mitochondria are dynamic membranous structures which continuously undergo scission and fusion. These processes are crucial for the maintenance of respiratory and metabolic activity and for the preservation of mitochondrial DNA. Malfunctioning of this process has been linked to numerous pathologies, such as neurodegenerative diseases, cardiomyopathy and cancer. Three dynamin-like proteins, dynamin-like-protein 1 (DNM1L), OPA1 (optical atrophy 1) and mitofusin are implicated in mitochondrial dynamics. Unlike in endocytosis, mitchondrial membrane fusion and division involves the double-membrane system of mitochondria. Consequently, mitochondrial dynamin-related GTPase act on distinct mitochondrial membranes, e.g. mitofusin mediates fusion of the outer mitochondrial membrane whereas OPA1 catalyzes fusion of the inner mitochondrial membrane.

We recently determined the crystal structure of the mitochondrial membrane scission protein DNM1L. Based on a range of biochemical and cell-based experiments, we deduced an alternative assembly mode of DNM1L at the outer mitochondrial membrane for the constriction of mitochondria. These results may have implications for mitochondria-based diseases in which DNM1L function is compromised.

Oligomerization and cellular function of Eps15 homology domain-containing ATPases (EHDs)

EHDs are ubiquitously expressed dynamin-related ATPases which are built of an

amino-terminal GTPase domain, followed by a helical domain and a carboxy-terminal regulatory Eps15 homology domain. EHDs are found at the plasma membrane and at vesicular and tubular endocytic membrane structures. They are implicated in several membrane trafficking pathways, most notably during the recycling of receptor proteins to the plasma membrane.

We previously showed that EHD2 oligomerizes in ring-like structures around tubulated liposomes. By solving the crystal structure of an EHD2 dimer, we found that stable dimerization of EHD2 is mediated via the GTPase domains. We identified the lipid-binding site at the tip of the helical domains. More recently, we showed that also the N-terminus of EHD2 can insert into the membrane, thereby regulating membrane recruitment and/or oligomerization of EHD2. Based on functional experiments, we suggested a model for the EHD2 oligomeric rings (Fig. 1c). These rings have a remarkably different architecture from those formed by MxA and dynamin. Eventually, we identified EHD2 as a novel integral component of caveolae opening up a wide range of cell-based approaches to study the physiological function of this protein.

GIMAPs - cellular scaffolds controlling lymphocyte survival

GTPase of the Immunity Associated Proteins (GIMAPs) comprise a septin-related GTPase family in vertebrates. The seven human GIMAP members are predominantly expressed in cells of the immune system. Some GIMAPs were proposed to regulate apoptosis by controlling the activity of Bcl2 proteins.

We determined four crystal structures of a representative member, GIMAP2, in different nucleotide-loading states. In combination with biochemical experiments, this work elucidated the molecular basis of GTP-dependent oligomerization via the GTPase domains (Fig. 1d). We also showed that GIMAP2 in Jurkat cells localizes to the surface of lipid droplets which are cellular storage and signalling compartments. Interestingly, GIMAP2 bound GTP with high affinity but did not hydrolyze it.

Using co-localization experiments, we recently identified GIMAP7 at the surface of lipid droplets. In contrast to GIMAP2, GIMAP7 efficiently hydrolyzed GTP. It also stimulated the GTPase activity of GIMAP2. The structure of a GTP-bound GIMAP7 dimer elucidated the mechanism of dimerization-induced GTPase activation. These data suggested that GIMAP7 acts as GTPase activating protein for GIMAP2. Furthermore, we found GIMAPs differentially down-regulated in a variety of different anaplastic large cell lymphoma lines compared to their T-cell progenitors, implicating a function of certain GIMAPs as tumor suppressors.

Selected Publications

Gao, S., von der Malsburg, A., Paeschke, S., Behlke, J., Haller, O., Kochs, G., and Daumke, O. (2010). Structural basis of oligomerization in the stalk region of dynamin-like MxA. Nature 465: 502-506.

Gao, S., von der, M.A., Dick, A., Faelber, K., Schroder, G.F., Haller, O., Kochs, G., and Daumke, O. (2011). Structure of myxovirus resistance protein a reveals intra- and intermolecular domain interactions required for the antiviral function. Immunity 35: 514-525.

Faelber, K., Posor, Y., Gao, S., Held, M., Roske, Y., Schulze, D., Haucke, V., Noe, F., and Daumke, O. (2011). Crystal structure of nucleotide-free dynamin. Nature 477: 556-560.

Fröhlich C., Grabiger S., Schwefel D., Faelber K., Rosenbaum E., Mears J., Rocks O., Daumke O. (2013) Structural insights into oligomerization and mitochondrial remodelling of dynamin 1-like protein. EMBO J 32: 1280-92.

Schwefel D., Arasu B.S., Marino S., Lamprecht B., Köchert K, Rosenbaum E.,Eichhorst J., Wiesner B, Behlke J., Rocks O., Mathas S., Daumke O. (2013) Structural insights into the mechanism of GTPase activation in the GIMAP family. Structure 21: 550-9

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Photo: David Ausserhofer/MDC

Bernd Dörken

Haematoloy, Oncology and Tumorimmunology

Biology and Targeted Therapy of Lymphoma

A detailed molecular understanding of the oncogenic process is fundamental for the development of new therapeutic strategies for human lymphomas. Using cell and molecular biology techniques, high-throughput approaches as well as in vivo mouse models, we aim to gain insight into the network of oncogenic defects in transformed B and T cells. In particular, we are interested in the link between deregulated signaling and transcription factor activities, disruption of cellular differentiation and oncogenic transformation. Using classical Hodgkin lymphoma (HL) and anaplastic large cell lymphoma (ALCL) as model systems, we analyze the role of lineage infidelity, cellular reprogramming and activation of lineage-inappropriate survival signals in lymphomagenesis, as exemplified by our work on the transcription factor E2A, which is essential for the development of B and T lymphoid cells. Complementary to studying lymphoma cell autonomous processes, we focus on the elucidation of lymphoma-stroma interactions. We have defined growth and survival niches for B cell lymphoma cells in secondary lymphoid organs, and we are further dissecting stromal cell subpopulations to determine essential signals that cause B cell lymphomas to be addicted to their local microenvironment. Collectively, our approaches do not only address fundamental aspects of leukemia and lymphoma pathogenesis, but they are also aimed at providing therapeutic solutions.

The role of deregulated transcription factor networks for differentiation programs, proliferation and survival of malignant lymphoma cells

M. Janz, S. Mathas, B. Dörken

Deregulated transcription factor (TF) activities are commonly observed in human lymphomas and leukemias, and the link between structural or functional alterations in TFs and malignant transformation has been documented in various in vitro and in vivo studies. Apart from the direct modulation of cellular processes like cellular growth or cell death, alterations of the activity of even single TFs might enforce malignant transformation by switching differentiation programs and consequently altering the cellular fate of the respective cells, as exemplarily demonstrated for the B lymphoid TF Pax5. A number of lymphoid tumors display a phenotype that is in accordance with such a reprogramming process, including HL, primary effusion lymphoma (PEL) and ALCL. Among these, cHL constitutes the most prominent example for lineage infidelity and reprogramming: in striking contrast to their origin from B cells, the malignant Hodgkin-/Reed-Sternberg (HRS) cells of cHL have almost completely lost their B cell-specific gene expression program and have acquired the expression of genes characteristic for other hematopoietic lineages. In our previous work, we could show that in particular the activity of TF E2A is severely disrupted in the malignant cells of HL and ALCL. We could furthermore demonstrate that this disruption is directly linked to a reprogramming process that results in the up-regulation of genes usually suppressed in these lymphoma cells, including genes normally expressed in myeloid cells such as the CSF1 receptor (CSF1R) gene. Remarkably, we observed that expression of CSF1R in HL cells is not mediated by the canonical myeloid promoter, but by an upstream long terminal repeat (LTR) that is aberrantly activated due to a loss of epigenetic control. Apart from these insights into the mechanisms of aberrant gene expression in lymphoma cells, our work has significant impact for the development of new treatment strategies. For example, inhibition of CSF1R activity by specific small compound inhibitors or multi-tyrosine kinase inhibitors like BAY 43-9006 resulted in blocked proliferation and enhanced cell death of lymphoma cells. Further investigations regarding the molecular reprogramming mechanisms, therapeutic implications and, in cooperation with the group of Klaus Rajewsky, the generation of transgenic mouse models are ongoing projects of our group.

Molecular defects in T cell-derived lymphoproliferative disorders

S. Mathas, M. Janz, B. Dörken

Apart from the identification of deregulated TF networks as outlined above, we are interested in the identification of molecular and genomic defects of T cell-derived lymphoproliferative disorders. In particular, we focus on the pathogenesis of ALCL and Sézary syndrome, a subtype of cutaneous T cell lymphoma. In ALCL, we have developed a concept for the generation of the ALCL-related chromosomal translocation t(2;5)(p23;q35). In this project we have already identified a number of deregulated genes involved in the dedifferentiation process as well as growth and survial of these cells. It is the aim of our ongoing work to investigate the mechanisms leading to the t(2;5)-translocation in ALCL and evaluate the consequences of breakpoint-proximal gene deregulation for prognosis and development of new treatment strategies for ALCL.

In a cooperation project with the Department of Dermatology of the Charité, Universitätsmedizin Berlin, we could identify the loss of TF E2A as a hallmark of Sézary syndrome (SeSy). Genomic loss of E2A is found in the majority of SeSy patient cases, and this loss is required for the uncontrolled growth of SeSy lymphoma cells. In addition, we have defined E2A-driven genes in SeSy tumor cells supporting a role of E2A as a tumor suppressor gene in human lymphoid cells. Interestingly, among E2A-regulated genes we identifed the oncogene MYC, which points to a direct link between the loss of differentiation programs and activation of oncogene activities. In line with the known requirement to counteract the MYC-driven induction of cell death, we have shown that the tumor sup-

pressor gene TP53 is lost in the majority of SeSy patient samples and non-functional in nearly all investigated lymphoma samples. Ongoing projects include the identification of further molecular and genomic defects as well as their impact on the development of new treatment strategies for these T cell-derived malignancies.

Tumor and microenvironment: Cellular and molecular prerequisites for onset and progression of leukemia and lymphoma

Armin Rehm and Bernd Dörken; in collaboration with Uta E. Höpken (MDC) and Georg Lenz (Charité-MKFZ)

A hallmark of human Hodgkin and non-Hodgkin lymphomas is their propensity to enter secondary lymphoid organs (SLO). Thus, two principal questions arise: what mechanisms regulate trafficking of lymphoma cells to SLOs and secondly, what selective growth advantage might those malignant lymphoid cells encounter in their niches.

Applying a Myc-driven B cell lymphoma model in mice, we have identified that the chemokine receptor CCR7 regulates access of lymphoma B cells to the T cell zone of SLOs (Rehm et al., Blood 2011). Lodging within this microanatomical niche launched a reciprocal crosstalk with fibroblastic reticular cells and DCs, a process triggered by the interaction of lymphotoxin-expressing lymphoma B cells and lymphotoxin β receptor-activated stromal cells. Subsequently, stromal cells released chemokines for tumor cell recruitment and growth factors for their protection from apoptosis.

Targeting the stromal environment is an appealing treatment option for lymphoma because this benign compartment is subject to low selective pressure for mutations and epigenetic changes compared with the tumor cells themselves.

The sorting receptor Sortilin has a dual function in effector molecule release from T and NK cells

Armin Rehm and Bernd Dörken; in collaboration with Uta E. Höpken (MDC), Gerald Willimsky (Charité, Institut für Immunologie), and Thomas Willnow (MDC)

One goal of our acitivities in tumor immunology is the development of an adoptive T cell therapy (ATT) that is highly effective and has low/no side effects. Prior to translating ATT to a broader range of cancer types, several immunological, clinical and technological obstacles must be overcome. Our contribution to this field is fueled by basic cell biological discoveries in T cells. Hence, we are addressing the question of how the secretory pathway in T cells regulates effector molecule release, e.g. perforin, granzymes and IFN-γ.

In recent work, we have shown that the sorting receptor Sortilin controls constitutive release of IFN-γ and granzyme A along the regulated secretory pathway (Herda et al., Immunity, 2012). In murine Sortilin-deficient CTLs and NK cells, regulated secretion of granzyme A and cytotoxic killing was enhanced and correlated with an increased availability of the SNARE-molecule VAMP7. In contrast, loss of Sortilin reduced the release of IFN-γ upon adenovirus or Listeria monocytogenes infections. From both infection models we infer that enhanced secretion of granzyme A cannot compensate for the strong reduction in IFN-γ release because liver damage caused by prolonged adenovirus persistence was stronger and titers for Listeria monocytogenes in livers of Sortilin-deficient mice were higher. Mechanistically, exit of IFN-γ from the Golgi apparatus required the presence of Sortilin, which acts as a sorting receptor for the inclusion of IFN-γ into clathrin-coated vesicles destined for endosomes. In contrast, other cytokines important for T cell differentiation and effector functions, such as IL-2 and TNF-α, remained unaffected. Furthermore, we tracked the transport route

of IFN-γ further downstream of the early endosome sorting step, demonstrating that IFN-γ is secreted independently from recycling endosomes and late endosomes. Our results suggest that Sortilin can contribute to the functional avidity of T cells through exocytic sorting of key effector molecules.

Selected Publications

Lamprecht*, B, Walter*, K, Kreher*, S, Kumar, R, Hummel, M, Lenze, D, Köchert, K, Bouhlel, MA, Richter, J, Soler, E, Stadhouders, R, Jöhrens, K, Wurster, KD, Callen, D, Harte, MF, Giefing, M, Barlow, R, Stein, H, Anagnostopoulos, I, Janz, M, Cockerill, P, Siebert, R, Dörken, B, Bonifer, C, Mathas, S. (2010). De-repression of an endogenous long terminal repeat activates the CSF1R proto-oncogene in human lymphoma. Nat. Med. 16, 571-579. *) equal contribution.

Steininger, A, Möbs, M, Ullmann, R, Köchert, K, Kreher, S, Anagnostopoulos, I, Hummel, M, Richter, J, Beyer, M, Janz, M, Klemke, CD, Stein, H, Dörken, B, Sterry, W, Schröck, E, Mathas, S, Assaf, C. (2011). Genomic loss of the putative tumor suppressor gene E2A promotes lymphoma in human. J. Exp. Med. 208, 1585-1593.

Calado, DP, Sasaki, Y, Godinho, SA, Pellerin, A, Köchert, K, Sleckman, BP, de Alborán, IM, Janz, M, Rodig, S, Rajewsky, K. (2012). The cell cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. Nat. Immunol. 13, 1092-1100.

Rehm, A., Mensen, A., Schradi, K., Gerlach, K., Winter, S., Büchner, G., Dörken, B., Lipp, M., Höpken, U.E., (2011). Cooperative function of CCR7 and lymphotoxin in the formation of a lymphoma-permissive niche within murine secondary lymphoid organs. Blood 118, 1020-1033.

Herda, S., Raczkowski, F., Mittrücker, H.-W., Willimsky, G., Gerlach, K., Kühl, A.A., Breiderhoff, T., Willnow, T.E., Dörken, B., Höpken, U.E.,Rehm, A. (2012). The sorting receptor Sortilin exhibits a dual function in exocytic trafficking of interferon-g and granzyme A in T cells. Immunity 37, 854-866

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Photo: David Ausserhofer/MDC

Iduna Fichtner

Experimental Pharmacology

In the recent years, our group has intensified in close collaboration with the Experimental Pharmacology and Oncology GmbH the accomplishment of the biobank with patient-derived xenograft (PDX) models (Figure 1). The collected samples have been used for the identification of biomarkers with a special focus on gene mutations. Further, the testing of novel targeted compounds has been continued within the framework of large grant-financed consortia. Alltogether, the PDX tumours have been regarded worldwide as the best available models for preclinical drug development because of their unlimited availability, their close relation to the clinical situation and the preservation of heterogeneity and complexity.

In the field of nanoparticle research we were focusing on the generation of vesicles for the treatment of brain tumours and metastases. The developed platform technology proved the general feasibility to overcome the blood brain barrier and can be used for the optimization of treatment of brain malignancies. Within our stem cell research we were interested in the establishment of standardized procedures for the preclinical monitoring of engraftment and differentiation with the aim to integrate the technology into risk assessment procedures for the use of embryonic stem cells.

Patient derived xenografts for the preclinical development of anticancer drugs

An extended panel of more than 400 PDX models has been collected and characterized in close cooperation with the Charité and several clinics in Europe representing the broad variety and heterogeneity of tumour tissue. Among that are large cohorts of specimen of colon (200), head & neck (46), renal (22) cancer and leukemias (47). Each PDX model represents an individual patient tumour (Figure 2) as correlation analyses at protein and gene level have revealed. The response rates of the PDX models clearly resemble the clinical situation both concerning standard of care treatments and novel targeted therapies. Genomic analyses of 67 colorectal carcinomas proved that mutations in KRAS, BRAF and PIK3CA clearly could discriminate between Cetuximab responder and nonresponder. It was shown in a case study of an individual patient that a complete remission of the tumour after Cetuximab treatment was observed despite of the presence of a KRAS mutation. That response was reflected in the clinics. We are now searching for biomarkers of that unexpected response in several colorectal PDX models with similar features. Extensive studies with PDX models of head & neck cancer confirmed the very individual response to clinically used agents like Docetaxel, 5-Fluorouracil, Carboplatin and Cetuximab. Microarray studies revealed a clear difference between Cetuximab responder and nonresponder in gene profile enabling the identification of relevant predictive biomarkers. Comparative mutational analyses proved a stable mutation profile between patient and mouse xenografted samples if the frequen-

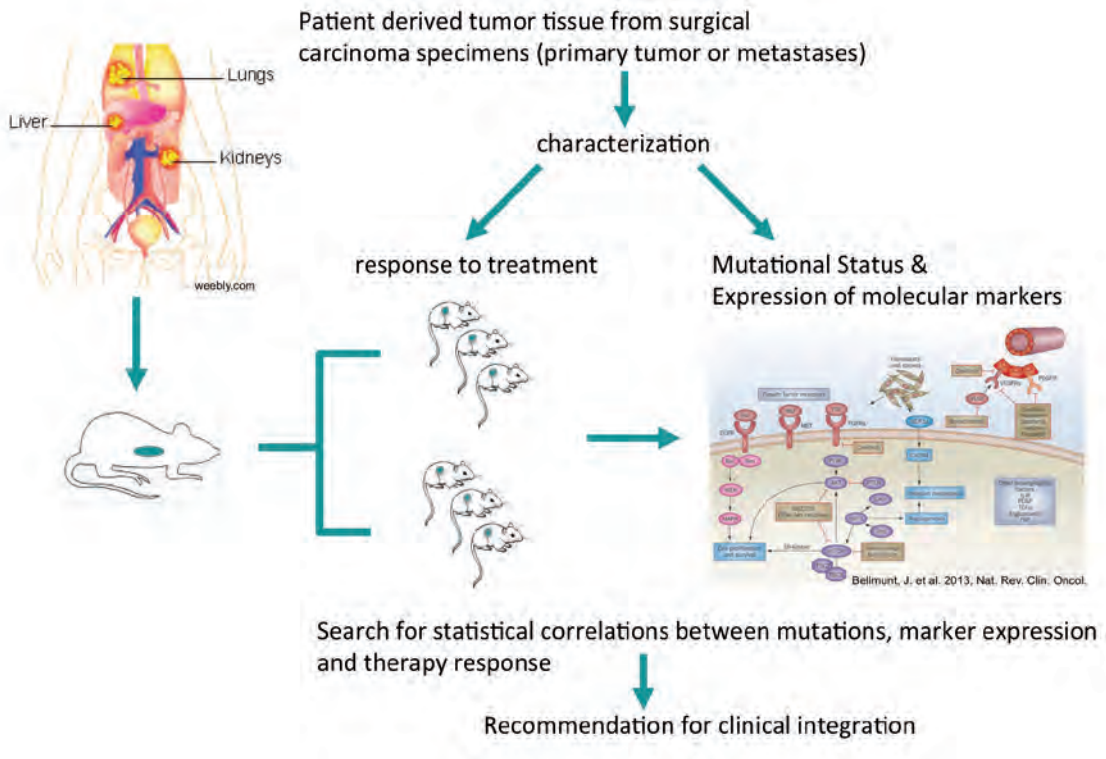


Figure 1: *Use of patient-derived xenografts (PDX) for the preclinical development of novel anticancer agents*

cy of the mutation was higher than 20%. Within the studies of renal cancer we were able to compare both biomarker expression and response data of several regions of the same tumour. The results confirmed the high intratumour heterogeneity making a successful treatment of cancer very challenging.

Development of nanoparticles for the treatment of brain malignancies

A systemic treatment of primary and secondary brain tumours still remains challenging because of the existence of the blood-brain barrier (BBB), preventing efficient drug passage.

In our previous work regarding liposomal drug transport of Mitoxantrone across the BBB we found that fluid membrane liposomes, targeted to the LRP receptor are suitable drug transporters for the treatment of experimental brain tumors. In the recent period, LRP-targeted and non-targeted liposomes were prepared to encapsulate oxaliplatin (OxP) and tested for tumour growth inhibition of human MT-3 breast cancer cells, transplanted both subcutaneously (s.c.) and into the brain of nude mice in order to demonstrate the potential of these liposomes to treat both the primary tumour and intracerebral metastases. It was demonstrated in a pharmacokinetic approach that the liposomes significantly increased Cmax and AUC in plasma compared to the free drug (3.5-4.5fold). Accumula-

tion of OxP in the s.c. growing tumors was enhanced up to 3.4fold. In the brain, both non-targeted and targeted liposomes accumulated better than the free drug (increase in AUC in comparison to free drug: 3,7fold or 2.9fold, respectively). In a therapeutic experiment, treatment with free OxP had no effect, while the liposomal formulations clearly inhibited the growth of brain tumours by 64% and 59% using non-targeted and targeted liposomes, respectively (Figure 3). The results further indicate that the liposomes developed in our group represent a platform technology for different drugs to improve a systemic treatment of brain malignancies.

Stem cell engraftment and differentiation

A potential application of stem cells in patients requires validated methods for the monitoring of engraftment and differentiation. We simulated that approach by using both human and murine stem cells in preclinical studies aiming at the development of standardised analytical methods. Live cell imaging by bioluminescence technique was applied to follow murine embryonic stem cell engraftment after transplantation via different application routes (s.c., i.splen., i.hep., i.v.). Distribution and organ engraftment were monitored in a time dependent manner. Differentiation of stem cells into germ layer progenitors was measured in the formed teratomas by histology, RT-PCR and immunofluorescence/immunhistochemistry. This technique was further qualified as an embryotoxicity test. Agents with known embryotoxic potential (none, weak, strong) were investigated in the validated teratoma model and cytotoxic activity could be quantified in the expected way. The established procedures were further used to investigate cell engraftment and distribution of human embryonic and

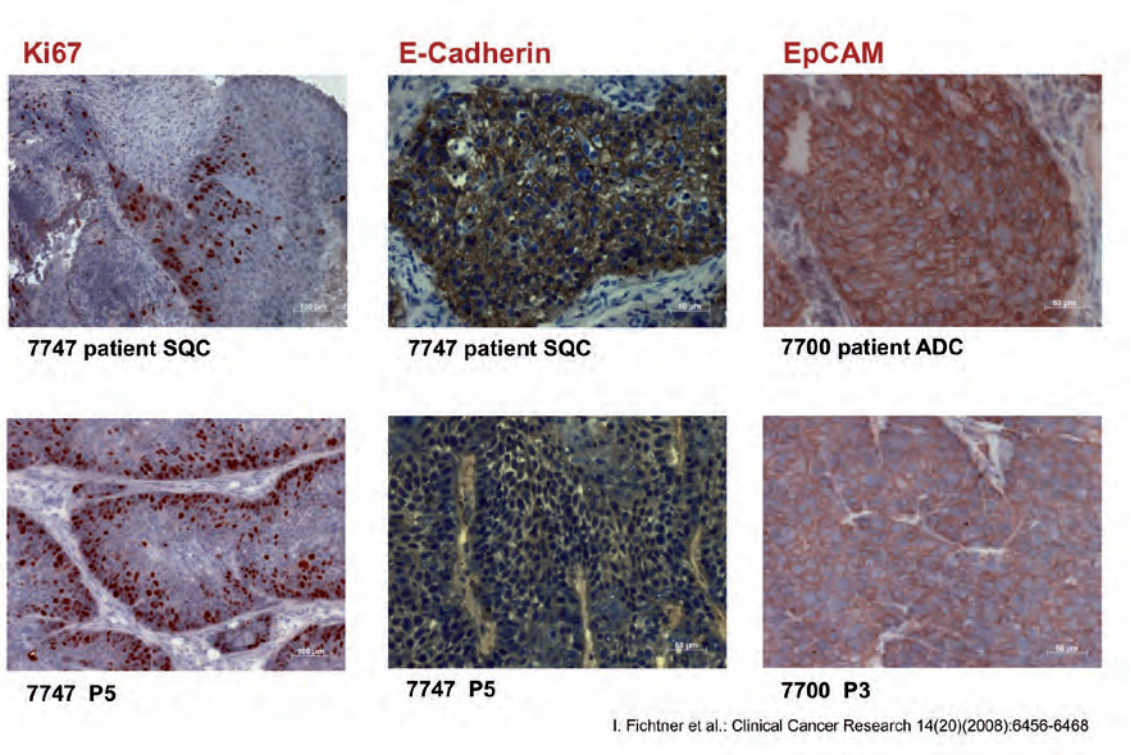


Figure 2: *Identity of immunohistological staining of patient non-small cell lung carcinomas (upper row) and the corresponding PDX models (lower row). SQC squamous cell carcinoma, ADC adeno carcinoma*

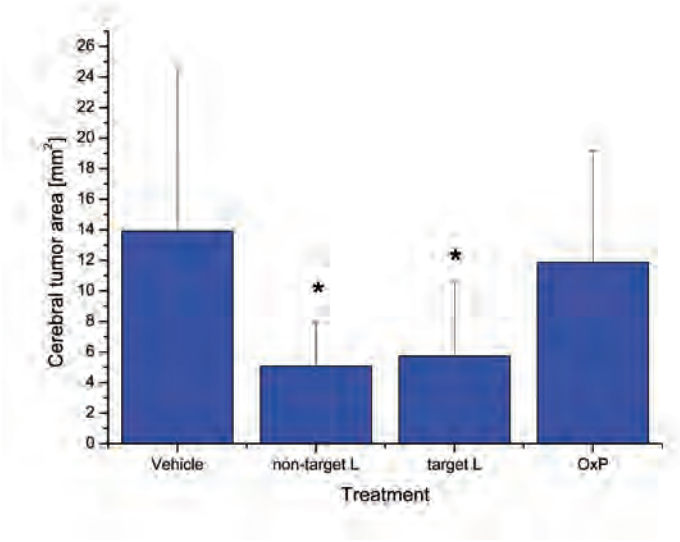


Figure 3: *Inhibition of intracerebral growing MT-3 tumours. Mice (n=6 per treatment arm) were treated with 6 mg/kg OxPt in free form (OxP), in non-targeted (nTL) and in targeted liposomes (TL) at day 3,7,12,17. At day 19 mice were sacrificed and the tumour area was measured.*significantly different to vehicle and OxP treated group ($p<0.05$).*

mesenchymal stem cells in cooperation with Charité and BCRT. We further established standard operating procedures allowing the characterization and the live cell imaging for testing the tumour formation and regenerative capacity of stem cells in experimentally injured organs. In addition to the research with embryonic stem cells we carried out studies with human hematopoietic stem cells. In vivo studies concerning the function of hematopoietic stem cells are limited due to low levels of engraftment and failure of the engrafted cells to differentiate into functional immune cells. Cord blood stem cells were transplanted into the liver of newborn and adult immunodeficient mice and the effect on organ engraftment and differentiation was analysed. A significantly high engraftment was found after transplantation into the liver of newborn NOD/SCID mice compared to adult mice, with the highest level of human cells in bone marrow and spleen. These human cells showed CD19 B-cell, CD34 and CD38 hematopoietic and CD33 myeloid cell differentiation, but lacked any T-cell differentiation. On contrary, human stem cell transplantation into the liver of adult NOD/SCID mice resulted only in minor recruitment of human cells in mouse liver and other organs. The results indicate the usefulness of the intrahepatic application route into the liver of newborn mice for the establishment of a human immune system with at least partial differentiation.

Selected Publications

Stachelscheid H, Wulf-Goldenberg A, Eckert K, Jensen J, Edsbagge J, Björquist P, Rivero M, Strehl R, Jozefczuk J, Prigione A, Adjaye J, Urbaniak T, Bussmann P, Zeilinger K, Gerlach JC. (2013). Teratoma formation of human embryonic stem cells in three-dimensional perfusion culture bioreactors. J Tissue Eng Regen Med. 7, 729-741

Wulf-Goldenberg, A., Keil M., Fichtner I, Eckert K. (2012). Intrahepatic transplantation of CD34+ cord blood stem cells into newborn and adult NOD/SCID mice induce differential organ engraftment. Tissue Cell. 44, 80-86

Pechańska P, Becker M, Mayr T, Hinzmann B, Adams H-P, Klaman I, Kretschmar K-H, Kretschmar K, Stecker K, Mantke R, Pauli R, Pertschy J, Hertel K, Ridwelski K, Hellwig K, Pross M, Radke C, Fichtner I, Hoffmann J, Rosenthal A. (2013). Mutation status of KRAS, BRAF, PIK3CA and expression level of AREG and EREG identify responders to cetuximab in a large panel of patient derived colorectal carcinoma xenografts of all four UICC stages. Journal of Cancer Therapy 4, 678-693.

Gries J, Schumacher D, Arand J, Lutsik P, Markelova MR, Fichtner I, Walter J, Sers C, Tierling S. (2013). Bi-PROF: Bisulfite profiling of target regions using 454 GS FLX Titanium technology. Epigenetics 8, 765-771.

Orthmann A, Zeisig R, Süss R, Lorenz D, Lemm M, Fichtner I. (2012). Treatment of experimental brain metastasis with MTO-Liposomes: Impact of fluidity and LRP-targeting on the therapeutic result. Pharm Res 29, 1949-1959.

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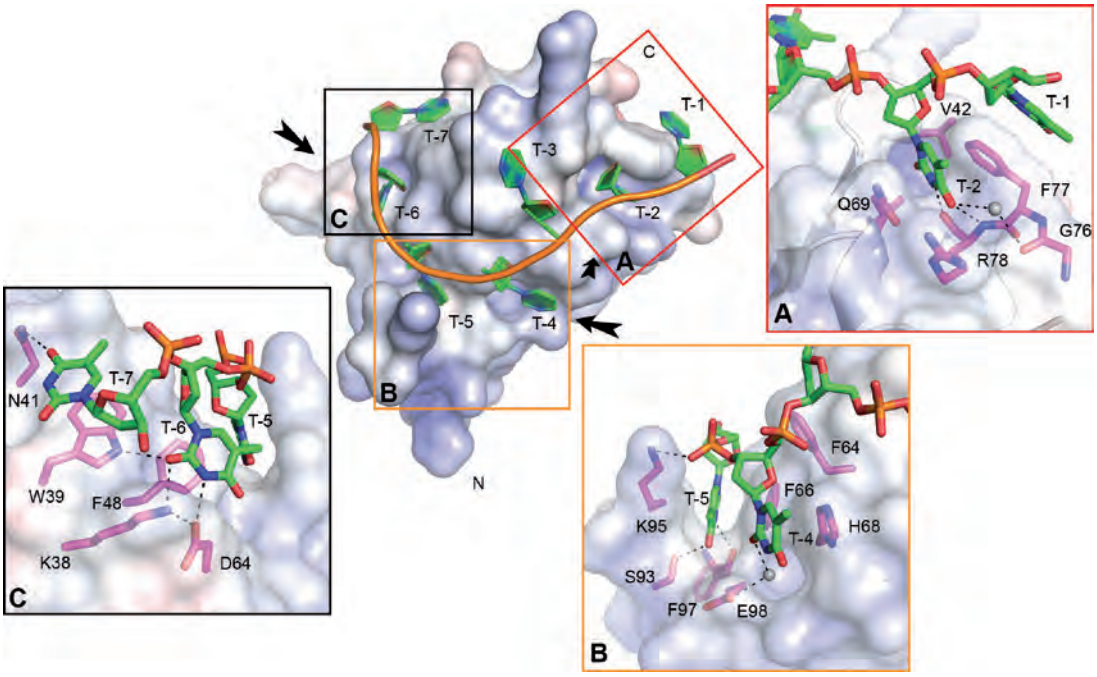
Macromolecular Structure and Interaction

Structural biology can reveal the molecular basis of cellular biology in exquisit detail and is uniquely powerful in generating testable hypotheses regarding molecular function. We are combining the core technique of structural biology, macromolecular crystallography, with biochemical and biophysical analyses to study the molecular basis of two different cellular processes. Firstly, we are interested in macromolecular interactions that govern the regulation of gene expression, both at the transcriptional and translational level. Secondly, we study the molecular machineries supporting intracellular transport processes. Here, we focus on two aspects, endoplasmic reticulum-associated protein degradation (ERAD) and vesicular transport. In addition, we are engaged in numerous collaborations with laboratories needing structural insight into biological problems of their choice. This collaborative work is facilitated by the Helmholtz Protein Sample Production Facility (PSPF). As part of the Joint Berlin MX Laboratory we are engaged at the macromolecular crystallography facility at the Berlin BESSY synchrotron to which we enjoy priviledged access.

Structural basis of gene expression regulation

It is increasingly recognizied that gene expression control is not limited to the regulation of transcription initiation. Instead, the maintenance of mRNA homeostasis by RNA-binding proteins and microRNAs appears to be of equal importance. Transcriptome-wide cross-linking studies have revealed the presence of up to 1000 RNA-binding proteins in human cells. Many of these proteins contain recurrent domains that mediate RNA binding. We have a long-standing interest in one of these structural modules, the cold-shock domain (CSD) which is present in thousands of different proteins across all kingdoms of life. In bacteria, CSDs usually occur in isolation in small proteins. They bind single-stranded DNA and RNA with limited sequence selectivity to a conserved surface that presents up seven nucleotide-binding subsites.

In eukaryotes, CSDs are often associated with natively unfolded polypeptide regions and rarely found together with other folded domains in the same protein. Lin28 contains a CSD that is followed by a zinc-knuckle domain (ZKD) and is thus an exception to this rule. LIN28 is up-regulated in embryonic tissue and down-regulated in differentiated cells. Up-regulation of LIN28 in adult tissue is correlated with carcinogenic transformation. Lin28 acts in a negative feedback loop with the let-7 microRNA which is present in large amounts in differentiated cells. This loop is maintained by the Lin28-mediated inhibition of let-7 maturation through blocking the processing of pri-let-7 by Drosha and of pre-let-7 by Dicer as well as by recruiting terminal uridylate transferases that mark the miRNA precursor for degradation. We are interested in describing this



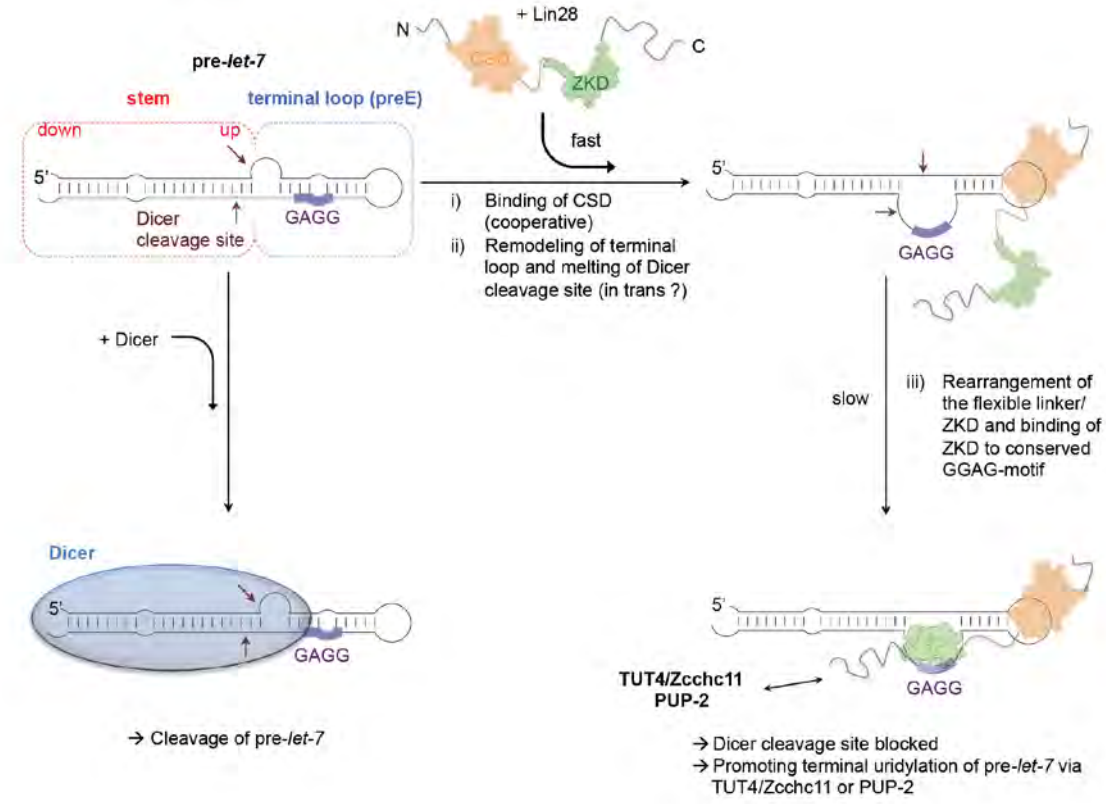
Binding of a DNA single strand to the cold-shock domain of Xenopus tropicalis Lin28b. *Center: The positively charged DNA- and RNA-binding surface of the cold-shock domain accommodates seven nucleotide residues which bind predominantly through stacking of nucleobases onto aromatic protein side chains. Sequence selectivity of binding is provided by several hydrogen bonds, some of which are water-mediated. Panels A-C: Detailed views of the subsites binding nucleosides T-1 and T-2 (A), T-4 and T-5 (B), and T-6 and T-7 (C). From Mayr et al. (2012).*

remarkable regulatory network in structural and biochemical terms.

To elucidate structural features of Lin28 binding to pre-let-7 miRNA, we produced various constructs of the protein ranging in length from the full-length polypeptide to the isolated CSD or ZKD. Crystal structure analysis revealed clear structural similarity of the Lin28 CSD with bacterial cold shock proteins. Binding of single-stranded oligonucleotides, in addition, followed the same pattern as in cold shock proteins (Figure 1). An analysis of RNA sequence preferences of Lin28 and the isolated Lin28 domains suggested that the CSD binds to the terminal loop of pre-let-7 whereas the ZKD requires a GGAG motif next to the terminal loop for binding. Importantly, we could show that the CSD by itself, but not the isolated ZKD is capable of pre-let-7 binding and inhibition of Dicer cleavage. It was therefore concluded that the Lin28 CSD is required for remodeling the let-7 precursor so that the ZKD is able to associate with its binding sequence (Figure 2). In further work we could contribute to a study of the Landthaler group of the MDC who showed that Lin28 binds a large number of other RNA sequences in addition to pre-let-7. With this laboratory we share a strong interest in other CSD-containing proteins such as the Y-box factors and in further RNA-binding proteins, notably RC3H1 whose crystal structure we could recently elucidate.

Structural aspects of intracellular transport

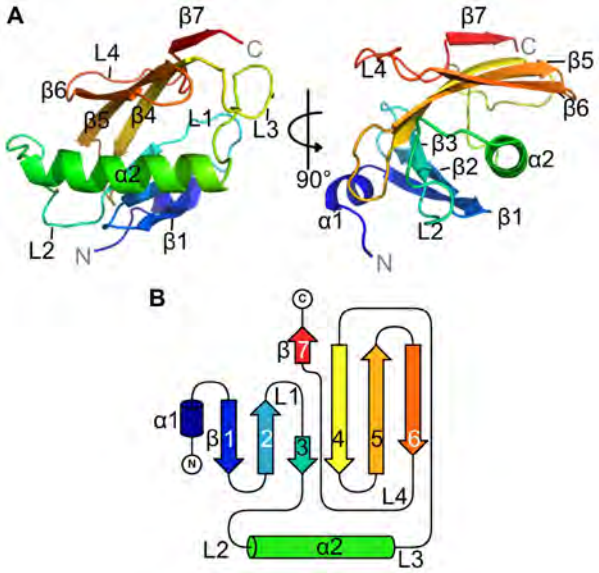
In the compartmentalized eukaryotic cell every molecule has to be moved to its site of activity. For proteins with functions outside the cytosol this necessitates their translocation across cellular mebranes. Membrane-enclosed vesicles are responsible for many steps in either endocytotic or exocytotic protein trafficking. In this context, we study the cellular machinery responsible for directing vesicles and establishing their initial physical contact to target mem-



Model of Lin28-mediated binding and inhibition of pre-let-7 maturation. *Our structural and biochemical analyses suggest that Lin28 binds the terminal loop (preE) of the let-7 precursor in a two-step process. Initial binding of the Lin28 cold-shock domain (CSD) to the terminal loop leads to a structural remodeling of preE that renders a GGAG sequence competent for tight and specific binding to the Zn-knuckle domain (ZKD) of Lin28. When both Lin28 domains occupy their sites on pre-let-7, cleavage by Dicer is blocked and maturation is inhibited. In addition, Lin28 recruits terminal uridylate transferases (TUT4/Zcchc11 or PUP-2) to pre-let-7 which mark the miRNA precursor for degradation. From Mayr et al. (2012).*

branes. Our work started with investigating structural aspects of the vesicular transport complex TRAPP (transport protein particle) which facilitates the tethering of COP II vesicles at the cis-Golgi membranes. The structures of several TRAPP subunits and sub-complexes were studied and served to elucidate architectural and functional aspects of TRAPP. Recently, this work was extended with the structure analysis of the yeast TRAPP-associated protein Tca17. Also, we have started to analyze structural features of the yeast HOPS (homotypic fusion and vacuole protein sorting) tethering complex which mediates the maturation of vacuoles from endocytic vesicles. Apart from multi-subunit tethering complexes, Rab GTPases and proteins with extended coiled-coil structures are important molecules in vesicle tethering. Our structural and biochemical analyses of human p115 provided evidence for a tetrameric arrangement of the protein supported by interactions in both its globular head and coiled-coil regions. Multiple Rab1 GTPase binding sites were mapped on the surface pf p115. The coiled-coil Golgi marker protein GM130 bridges between p115 and the PDZ domains of the membrane-anchored protein GRASP65, a process for which we also provided structural insight.

Proteins entering the exocytotic route are co-translationally channeled to the lumen of the endoplasmic reticulum (ER) where they assume their native fold and undergo post-translational modification. Folding-incompetent luminal proteins are cleared from the ER in a process called ER-associated degradation (ERAD) which is studied in detail by the Sommer group of the MDC. The luminal lectin Yos9 of yeast is associated to the HRD ubiquitin-ligase complex, a central functional module of ERAD. We determined the crystal structure of the central domain of Yos9 (Figure 3) and showed that this domain is responsible for the formation of Yos9 dimers in solution. We could thus provide evidence for a dimerization interface within the ER lumen that is likely to sup-



Structure of the dimerization domain of the ERAD component Yos9, a lectin associated to the HRD ubiquitin ligase complex. *The central dimerization domain (DD, amino acids 266-422) of the ER luminal protein Yos9 is dimeric in the crystal and in solution. The dimerization interface comprises residues anchored in β-strands β5, β6 and β7. Site-directed mutagenesis of interface residues abrogates dimerization of both full-length Yos9 and Yos9 DD. Identification of a Yos9 dimer supports the model of a dimeric HRD-ligase complex which is further stabilized by protein-protein contacts within the ER membrane and in the cytosol. From Hanna et al. (2012).*

port a dimeric structure of the HRD ligase complex in conjunction with similar dimerization sites on the surface of HRD subunits located in the membrane bilayer or the cytosol. The cellular ERAD pathways are well conserved between yeast and mammals, but the lectin Yos9 has two human homologs, OS-9 and XTP3B. We have recently generated structural data for XTP3B which shed further light on the function of these proteins in ERAD substrate recognition and recruitment.

The cytosolic ERAD component Cdc48 of yeast is involved in substrate retro-translocation to the cytosol and delivery of polyubiquitinated protein chains to the proteasome for degradation. Its ortholog, the AAA+ ATPase p97 (or VCP for valosin-containing protein) is among the most abundant proteins in human cells. In addition to ERAD, p97 plays important roles in homotypic membrane fusion, DNA repair and as an inhibitor of apoptosis. p97 is a hexameric molecular machine whose activity is regulated by binding of adaptor proteins, many of which contain ubiquitin-X regulatory (UBX) domains. In close collaboration with the Wanker laboratory of the MDC we have determined crystal structures of complexes of p97 with the UBX adaptor ASPL (alveolar soft-part sarcoma locus). These complexes show a very unexpected structure and stiochiometry which has far-reaching functional implications.

Protein Sample Production Facility supports collaborative research

The Helmholtz Protein Sample Production Facility (PSPF) led by Anja Schütz is dedicated to the production of proteins for structural and biophysical studies. The PSPF provides a platform through which many groups from the MDC and beyond have access to crystallization-ready protein samples. In cases of mutual interest, this service can be extended to biophysical and structural characterizations of proteins and protein complexes. In recent years, MDC laboratories from all research programs have made use of the PSPF services.

Selected Publications

Hanna, J., Schütz, A., Zimmermann, F., Behlke, J., Sommer, T. & Heinemann, U. (2012) Structural and biochemical basis of Yos9 protein dimerization and possible contribution to self-association of 3-hydroxy-3-methylglutaryl-coenzyme A reductase degradation ubiquitin-ligase complex. J. Biol. Chem. **287**, 8633-8640.

Mayr, F., Schütz, A., Döge, N. & Heinemann, U. (2012) The Lin28 cold shock domain remodels pre-let-7 microRNA. Nucleic Acids Res. **40**, 7492-7506.

Sachs, R.*, Max, K.E.A.*, Heinemann, U.# & Balbach, J.# (2012) RNA single strands bind to a conserved surface of the major cold shock protein in crystals and solution. RNA **18**, 65-76.

Mayr, F. & Heinemann, U. (2013) Mechanisms of Lin28-mediated miRNA and mRNA regulation - A structural and functional perspective. Int. J. Mol. Sci. **14**, 16532-16553.

Seul, A.*, Müller, J.J.*, Andres, D., Stettner, E., Heinemann, U.# & Seckler, R.# (2014) Bacteriophage P22 tailspike: Crystal structure of the complete protein and function of the interdomain linker. Acta Crystallogr. D, in press.

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Photo: David Ausserhofer/MDC

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Cell Differentiation and Tumorigenesis

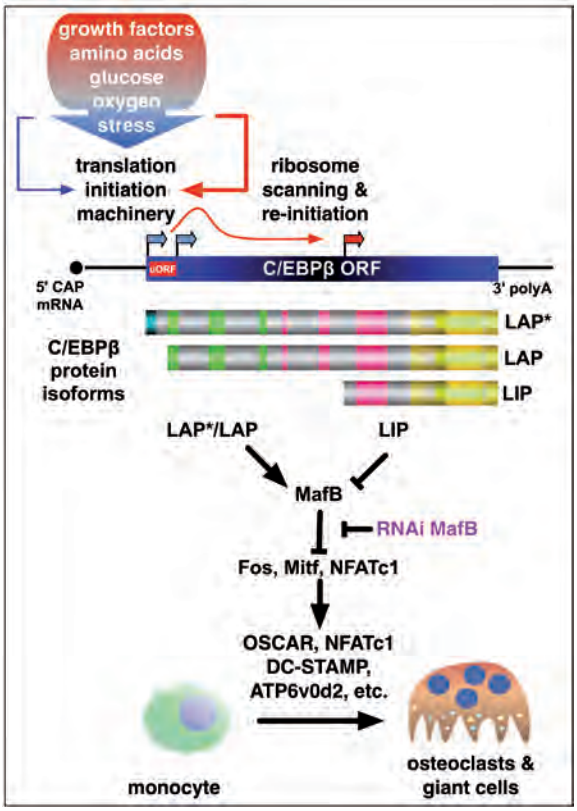
Self-renewal of somatic stem cells and differentiation into functional cells are epigenetic events that are initiated by transcription factors downstream of signaling cascades. Self-renewal vanishes with cell differentiation but may recur in tumor stem cells. Understanding the molecular mechanisms involved is central to the application of stem cell based bio-medicine and to the identification of the "Achilles' heel" in tumorigenesis. We study transcription factors of the CCAAT Enhancer Binding Protein (C/EBP) family that are key players in cell fate decisions in many organs. Somatic "stemcellness" is often regulated by the Wnt-β-catenin signaling pathway. We have explored the function of β-catenin in hematopoiesis and leukemogenesis.

Structure & Function of C/EBP

C/EBP transcription factors regulate functions in stem cells and differentiating cells and are involved in tumor formation. C/EBPs harbor a strong trans-differentiation and cell lineage instructive potential. Our research is focused on the dynamic regulation of C/EBPs and how these factors balance self-renewal versus cell differentiation.

C/EBPα,β,δ,ε, are highly related family members and probably the result of gene quadruplication in early vertebrate evolution. The C/EBPs display modular structures of conserved short amino acid regions that are intervened by low-complexity regions, suggesting structural flexibility. The N-termini harbor strong trans-activation domains (TAD) whereas the center sequences represent regulatory domains (RD). The C-terminal basic DNA binding leucine zipper domains (bZip) determine homo- and hetero-dimerization with other transcription factors and interaction with cis-regulatory DNA sequence motifs.

Compound gene deletion studies in the mouse revealed that C/EBPα and β are essential for tissue formation and homeostasis. Both, C/EBPα,β are single exon genes. Nevertheless, various C/EBP isoforms are generated by alternative translation initiation and by a multitude of post-translational amino acid side chain modifications (PTM). Both types of modifications are downstream of signaling events. They determine the C/EBP-interactome and play key roles in the orchestration of diverse C/EBP functions.



Translational regulation of C/EBPβ and osteoclast formation. *C/EBPβ translation is regulated by an upstream open reading frame (uORF) that senses the activity of the translational machinery and directs initiation to alternative start sites. The resulting isoforms, termed LAP*, LAP, and LIP, have distinct gene regulatory and epigenetic potential. The ratio between the isoforms determines expression of the key regulatory MafB protein in osteoclasts and giant cells. MafB in turn regulates several factors involved in cell fusion and differentiation.*

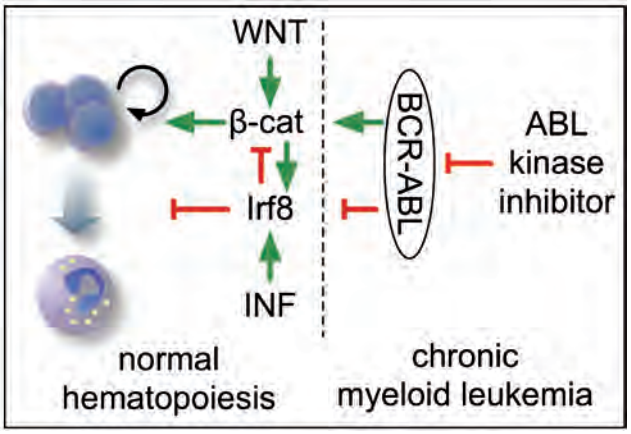
Translational regulation of C/EBP

The C/EBPα and C/EBPβ protein isoforms of different N-terminal lengths (termed LAP*, LAP, and LIP) are generated by alternative translation initiation (Figure 1). Both, C/EBPα and C/EBPβ mRNAs harbor small upstream open reading frames (uORF) that sense the activity of the translation initiation machinery and relay translation starts to alternative in-frame initiation sites. High activity of the translation machinery favors generation of truncated proteins, which lack part of the N-terminus, whereas lower translation activity favors long C/EBP isoforms. Using targeted genetics we have generated knockin mice that can not switch between C/EBPβ isoforms but express either only the long (LAP*, LAP) or the truncated C/EBPβ isoform (LIP). These mouse strains are susceptible to many diseases, including cancer, defects in bone homeostasis, liver regeneration, mammary gland development, and metabolism. For example, constitutive expression of truncated LIP isoform enhances the formation of multi-nucleated osteoclasts and induces bone diseases. Profiling of the LIP-mouse osteoclast transcriptome revealed MafB as a C/EBPβ target and as an inhibitor of osteoclastogenesis (Smink et al. 2009, EMBO J. 28:1769-81). These results suggest that interference with translational initiation may represent a potential target for treatment of degenerative bone diseases.

In contrast to the LIP mouse strain, mice deficient for the C/EBPβ uORF initiation codon (C/EBPβΔuORF) fail to initiate translation of the truncated C/EBPβ LIP isoform, but express the long isoforms LAP*/LAP. C/EBPβΔuORF mice are deficient for osteoclastogenesis, liver regeneration, and branching morphogenesis in the mammary gland. C/EBPβΔuORF mice display hyperactive acute phase response and persistent repression of E2F-regulated genes (Wethmar et al, 2010, Genes & Dev., 24:15-20). In summary, switching between C/EBPβ isoforms is important in regeneration, cell differentiation, and prevention of cancer.

Post-translational C/EBP modifications provide an index of epigenetic functions

Several years ago, we found that C/EBPα and β may instruct expression of myeloid genes in many cell types, even in skin fibroblasts. Accordingly, C/EBPs entail the epigenetic competence to orchestrate cell fate. Considering the modular structural/functional C/EBP features and seeking for an explanation of cell fate determination, it came to our attention that several arginine (R) and lysine (K) residues were targets for post-translational modifications (PTM) and that occurrence of PTMs depends on signaling events. Using a systematic mass spectrometric analysis (in collaboration



Connection between Wnt and interferon signaling in hematopoiesis and leukemogenesis. *Wnt signaling induces expression of Irf8 that inhibits expansion of granulocytic precursor cells and limits Wnt pathway functions. In the absence of Irf8 a chronic myeloid leukemia phenotype develops. Loss of Irf8, together with excessive Wnt signaling, causes acute leukemia. The human Philadelphia chromosome BCR-ABL oncoprotein suppresses Irf8 and enhances Wnt signaling, suggesting both pathways as key events in the formation of leukemic stem cells.*

with Gunnar Dittmar, MDC), we identified many novel modifications on C/EBPα and β, including K-acetylation and K-, R-methylation. Biochemical and molecular genetic analysis revealed that e.g. methylation of N-terminal C/EBPβ R3 abrogated interaction with both, SWI/SNF and Mediator complexes and that activation of ras-MAPK signaling inhibited methylation of R3 by PRMT4/CARM1 and of K39 by G9a. Moreover, mutagenesis of R3 and K39 enhanced gene activation. These data imply the existence of signal-dependent, intra-molecular crosstalk and modification directed interactions between C/EBPβ and the epigenetic machinery (Leutz et al, 2011, Transcription, 2:3-8).

An assay that converts primary mouse B lymphoid progenitors into myeloid cells was employed to examine how C/EBPβ directs alternative cell fates. The results showed that various C/EBPβ isoforms and PTMs define the outcome of trans-differentiation into different types of monocytes/macrophages, dendritic cells, and granulocytes. For example, a region in C/EBPβ was identified that, in conjunction with the bZip domain, is necessary and sufficient to induce myeloid-lymphoid conversion. Moreover, the ability of C/EBPβ to recruit chromatin remodeling complexes controls alternative trans-differentiation into macrophages and granulocytes (Stoilova et al., 2013, PLoS ONE, 8:e65169). These findings revealed that PTMs and the structural plasticity of C/EBPβ are adaptable modular properties that integrate and rewire epigenetic functions to direct differentiation into diverse innate immune cells.

Observations of trans-differentiation were initially regarded merely as the result of artificial manipulation, with no physiological correspondence. However, incoherent cell differentiation states were reported to emerge in pre-neoplastic tissue e.g. as epithelial metaplasia and 'lineage infidelity' in leukemia/lymphoma, echoing results from experimental cell reprogramming. Both, metaplasia and biphenotypic leukaemia/lymphoma may represent examples of dysregulated cell differentiation that reflect a history of trans-differentiation and/or epigenetic reprogramming. Studies are underway to determine the C/EBP interaction partners involved in reprogramming to elucidate the mechanism of trans-differentiation and to address the question whether reprogramming is involved in tumorigenic transformation (Regalo & Leutz, 2013, EMBO Mol Med, 5:1154-64) .

We surmise the existence of a post-translational "Indexing Code" on C/EBPs that may direct distinct protein interactions. The functional consequences of individual C/EBP-PTMs are difficult to discern and require the knowledge of differentially interacting partners for further functional investigations. We therefore employ high-end mass spectrometry and a systematic differential proteomic screening approach to detect PTM-dependent interactors. Our screens revealed epigenetically important proteins that differentially bind to distinct C/EBP isoforms or C/EBP-PTMs. We ex-

plore the possibility that the modifications on C/EBPs orchestrate co-factor interactions in order to direct the gene regulatory and epigenetic machineries in a gene context specific fashion. The multiplicity of C/EBP modifications comprise an extraordinarily high number of combinatorial interactions that mediate functional and evolutionary plasticity. We anticipate that unraveling the PTM-dependent C/EBP interaction network might help to elucidate complex functions in cell differentiation, regeneration, and tumorigenesis.

Crosstalk between Wnt- and interferon pathways in hematopoiesis & leukemia

Chronic myeloid leukemia (CML) is a clonal myeloproliferative stem cell disorder resulting from the stable recurrent Philadelphia chromosomal translocation in hematopoietic stem cells that gives rise to the oncogenic BCR-ABL fusion protein. Administration of BCR-ABL kinase inhibitors (e.g. Imatinib) can efficiently restrain CML, but complete remission is difficult to achieve due to persistence of inhibitor resistant leukemia initiating stem cells that may re-establish CML and cause relapse of disease. CML eventually progresses to a fatal blast-crisis phase that is characterized by accumulation of differentiation-arrested and therapy resistant blast cells, highlighting the need to understand the emergence of leukemia initiating cells.

Using mouse genetics and a BCR-ABL-model of CML, we observed crosstalk between Wnt/β-catenin signaling and the interferon (INF)-inducible transcription factor Irf8 (Figure 2). In normal hematopoiesis, activation of β-catenin results in up-regulation of Irf8, which in turn limits oncogenic β-catenin functions. In BCR-ABL CML cells, Irf8 is suppressed and β-catenin tends to be activated. Irf8-deficient animals develop a CML-like disease and self-renewal of hematopoietic stem cells and myeloproliferation becomes dependent on β-catenin. Combined deletion of Irf8 and constitutive activation of β-catenin results in progression of CML into fatal blast crisis, elevated leukemic potential and resistance to BCR-ABL inhibitors. Thus, crosstalk between Wnt and IFN signaling represents a BCR-ABL-independent mechanism of disease progression and establishes development of leukemia initiating cells. On the molecular level, activated β-catenin enhances a pre-existing Irf8-deficient gene signature, identifying β-catenin as an amplifier that causes progression of disease and the shift of CML into blast crisis. Since elimination of β-catenin did not affect normal hematopoietic stem cells, and since Irf8 antagonized BCR-ABL-induced leukemia, targeting of both pathways together with BCR-ABL inhibitor treatment may pave the way to more effective combinatorial therapeutic strategies in the treatment of CML.

Selected Publications

Kowenz-Leutz, E., Pless, O., Dittmar, G., Knoblich, M., and Leutz, A. (2010). Crosstalk between C/EBPbeta phosphorylation, arginine methylation, and SWI/SNF/Mediator implies an indexing transcription factor code. The EMBO J., 29, 1105-1115.

Leutz, A., Pless, O., Lappe, M., Dittmar, G., and Kowenz-Leutz, E. (2011). Crosstalk between phosphorylation and multi-site arginine/lysine methylation in C/EBPs. Transcription 2, 3-8.

Pless, O., Kowenz-Leutz, E., Dittmar, G., and Leutz, A. (2011). A differential proteome screening system for post-translational modification-dependent transcription factor interactions. Nature Protocols 6, 359-364.

Scheller, M., Schonheit, J., Zimmermann, K., Leser, U., Rosenbauer, F., and Leutz, A. (2013). Cross talk between Wnt/beta-catenin and Irf8 in leukemia progression and drug resistance. The Journal of Experimental Medicine 210, 2239-2256.

Wethmar, K., Begay, V., Smink, J.J., Zaragoza, K., Wiesenthal, V., Dorken, B., Calkhoven, C.F., and Leutz, A. (2010). C/EBPbetaDeltauORF mice: a genetic model for uORF-mediated translational control in mammals. Genes & Development 24, 15-20.

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Photo: David Ausserhofer/MDC

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Molecular Tumor Genetics and Immunogenetics

The chemokine system controls lymphocyte recirculation in immune system homeostasis as well as the activation-dependent and tissue-selective trafficking of lymphocytes and dendritic cells during immune responses. Our main focus is the role of homeostatic chemokine receptors like CXCR5 and CCR7 in lymphoid organ development, systemic immune responses, cancer, and chronic inflammatory diseases. We are also interested in the immune modulatory and growth-inducing functions of chemokine receptors encoded by human herpesviruses. In our translational program we have developed efficient recombinant and bispecific antibodies targeting B cell Non-Hodgkin lymphoma and multiple myeloma.

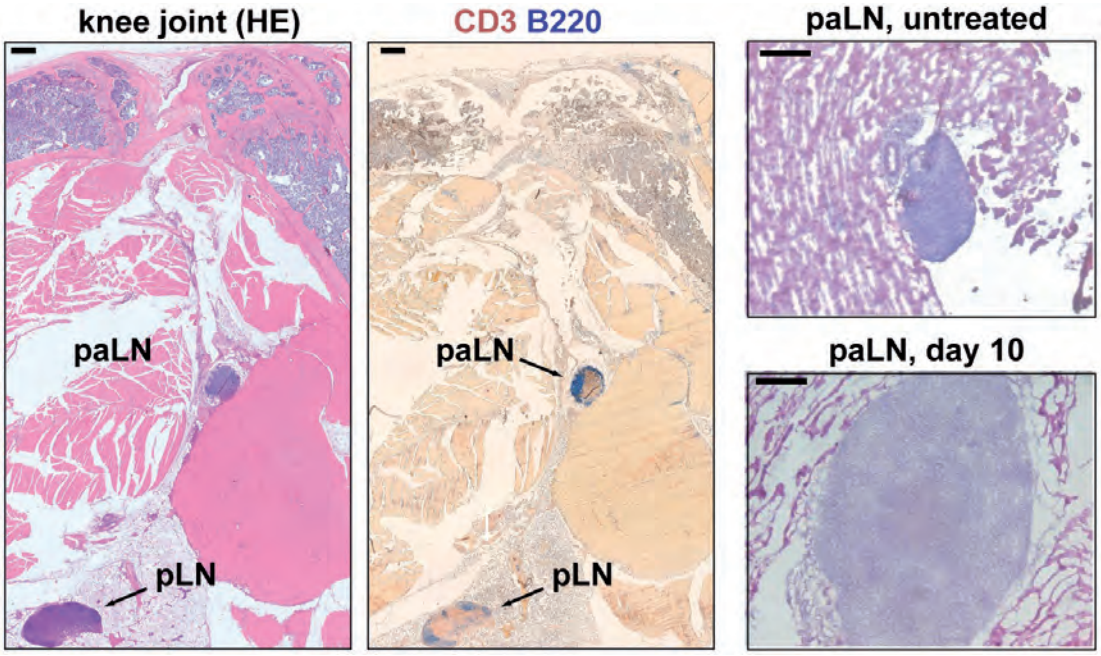
Differentiation and transcriptional control of follicular B helper T cells (T_{FH})

T cell help to B cells is essential to the development of B cells into antibody-secreting plasma cells and of immunological memory. B cell help is mediated by a specialized subset of CD4 T cells, called follicular T helper (T_{FH}) cells. Cognate interaction of antigen presenting B cells with T_{FH} cells at the edges of follicles in secondary lymphoid organs enables the B cells to enter B cell follicles. T_{FH} cells are also required during the germinal center reaction, when class switch recombination and affinity maturation occur, as they control multiple steps of B cell differentiation into high-affinity antigen presenting plasma cells or long-lived memory B cells. We could show by large-scale gene expression profiling that B helper cell activity is largely confined to CD4 T cells co-expressing the chemokine receptor CXCR5, which guides these cells into B cell follicles, and costimulatory molecules of the CD28 family such as the inducible costimulator ICOS. We have demonstrated that a transcription factor, that has long been considered a B cell-specific factor, promotes the development of T_{FH} cells. The role of this factor in T cells has long been overlooked as its function is masked in mice deficient for this factor by multiple defects in many stages of B cell development. Therefore, the most important outcome of our study was the finding that the identified transcription factor operated in a T cell intrinsic manner to control gene expression in T_{FH} cells. Without this factor, we observed major changes in the expression of costimulatory molecules on T_{FH} cells as well as a dramatic reduction in the frequency of T_{FH} cells.

Follicular regulatory T cells (T_{FR}) control humoral autoimmunity via NFAT2-regulated CXCR5 expression

(collaboration: M. Vaeth/F. Berberich-Siebelt, Department of Molecular Pathology, Julius-Maximilians-Universität, Würzburg)

Maturation of high affinity B lymphocytes is precisely controlled during the germinal center reaction. This is dependent on $CD4^+CXCR5^+$ T_{FH} cells and inhibited by $CD4^+CXCR5^+Foxp3^+$ follicular regulatory T cells (T_{FR}). NFAT2 was found to be highly expressed and activated in follicular T cells. Unexpectedly, ablation of NFAT2 in T cells caused an augmented GC reaction upon immunization. Consistently, however, T_{FR} cells were reduced in the follicular T cell population due to impaired homing to B cell fol-



Healthy BALB/c mice exhibit a unique periarticular lymph node (paLN) near the knee joint *(Left panel): Section of paraffin-embedded hind leg from BALB/c mouse in the early chronic phase of ACIA with hematoxylin eosin staining. Arrows: periarticular lymph node (paLN) close to the knee joint and popliteal lymph node (pLN) embedded in fat tissue. Black bars, 400 mm. (Middle panel): paLN and popliteal lymph node stained for B cells (B220, blue) and T cells (CD3, red). (Right panel): Ten days into the development of chronic arthritis, the paLN expands to a huge size (bottom right). [Nat. Commun. 2013;4:1644. DOI: 10.1038/ncomms2625]*

licles. This was T_{FR}-intrinsic since only in these cells NFAT2 was essential to upregulate CXCR5. The physiological relevance for humoral (auto-) immunity was corroborated by exacerbated lupus-like disease in the presence of NFAT2-deficient T_{FR} cells.

Transition from an autoimmune-prone state to fatal autoimmune disease

(Uta E. Höpken; collaboration: M. Schumann, B. Siegmund, A. Fischer, M.M. Heimesaat and S. Bereswill, Charité)

Autoimmunity is associated with a strong genetic component, but onset and persistence of clinically apparent autoimmune diseases often require an additional environmental trigger. The balance between immunity and tolerance is regulated by molecular factors including nuclear hormone and homeostatic chemokine receptors. The nuclear hormone receptor RORγt and the chemokine receptor CCR7 are both essentially involved in functional lymphoid organogenesis and maintenance of lymphocyte homeostasis. Lack of one or the other impairs thymic T cell development and alters T cell homeostasis. CCR7-deficient mice revealed an immune-mediated condition, which caused a dosed immune response of pro-inflammatory cytokines resulting in the development of autoimmune gastritis and chronic diarrhea. Mice deficient for both, $Ccr7^{-/-}Ror\gamma t^{-/-}$, succumbed early to acute destructive inflammation, characterized by massive recruitment of inflammatory leukocytes, pro-inflammatory cytokine and autoantibody production, and wasting disease. Antibiotic-treatment of mice before disease onset reduced the overall gut microflora and abrogated the development of fatal mucosal inflammation. Hence, the presence of commensal gut bacteria crucially contributes to this condition which eventually facilitates transformation of a non-life threatening autoimmune condition, as observed in the single-deficient $Ror\gamma t^{-/-}$ or the $Ccr7^{-/-}$ mouse strain, into a rapidly developing lethal autoimmune disease in $Ccr7^{-/-}Ror\gamma t^{-/-}$ mice.

Development of a novel chronic murine model of rheumatoid arthritis

Rheumatoid arthritis (RA) is a common autoimmune disease with unknown etiology that affects around 1% of the population. In RA, chronic inflammation of the synovium of diarthrodial joints leads to irreversible joint damage, which results in chronic pain and disability. Current animal models of RA have fundamental limitations as they are

lacking the complexity of the human disease. Among the restrictions is the failure of most models to induce the formation of RA-specific autoantibodies like ACPA, the rare development of an auto-antigen driven chronic course of the disease, and the restriction to few susceptible strains. Therefore, we developed a novel murine arthritis model by combining classical antigen-induced arthritis (AIA) and the collagen-induced arthritis (CIA) model. The rationale behind this set-up was to mimic a chronic disease associated with autoantibody production as observed in human RA in commonly used mouse strains such as BALB/c and C57BL/6. In BALB/c mice, the novel antigen- and collagen-induced arthritis (ACIA) model leads to a profound synovitis and strong cartilage and bone erosion. Moreover, it is characterized by an efficient and rapid formation of high serum levels of IgG against cyclic citrullinated peptides (CCP) and collagen II. Concomitant to the observation of a more severe pathology, we discovered a previously undescribed small periarticular lymph node in close proximity to the knee joint of BALB/c mice, which acts as the primary draining lymph node for the synovial cavity. Our model more closely reflects the pathology of rheumatoid arthritis than classical models and is hence particularly suitable for further studies.

Cellular and molecular dissection of the lymphoma-stroma in B cell lymphoma

(Uta E. Höpken, collaboration: A. Rehm/B. Dörken, MDC, Charité)

The crosstalk between lymphoid tumor cells and their environment provides pivotal signals for the initiation and progression of lymphoma. Histomorphological and immunophenotypical aspects of lymphoma recapitulate features characteristic of benign lymphoid neogenesis. We investigated the cooperation between homeostatic chemokine receptor-controlled dissemination and lymphotoxin (LT)-promoted niche formation in a transgenic mouse model of Myc-driven aggressive B cell lymphoma. We showed that CCR7 regulates Eμ-Myc lymphoma homing to lymph nodes and distinctive microanatomic sites of the spleen. CCR7-controlled access of lymphoma cells to the splenic T cell zone led to a survival advantage compared to CCR7-deficient lymphoma cells which were excluded from this zone. Within the niche, lymphoma cells stimulated a reciprocal crosstalk with $gp38^{+}$ fibroblastic reticular cells. This reciprocal cooperation program was mediated by lymphoma B cell-presented LT which acted on LTβR-bearing stromal cells followed by alteration of stromal cellular composition. Crosstalk inhibition by LTα deletion and by employing a LTβR -immunoglobulin fusion protein impaired lymphoma growth. Our aim is the dissection of cellular requirements and molecular pathways which contribute to the transformation of secondary lymphoid organs toward lymphoma-permissive niches.

A viral chemokine receptor (vGPCR)-triggered animal model reveals epigenetic mechanisms in immune escape and tumor progression

(collaboration: Wei Chen/G. Dittmar, MDC)

The human herpes virus 8-encoded G protein-coupled chemokine receptor (vGPCR) has been implicated in the pathogenesis of Kaposi´s sarcoma (KS) particularly because of its high constitutive signaling activity. We have used retroviral transduction to generate vGPCR-transformed BALB/c-3T3 fibroblasts that are tumorigenic in nude mice, but as expected fail to induce tumors in their immunocompetent counterparts. However, tumor fragments obtained from nude mice grow progressively in BALB/c mice. Unexpectedly, vGPCR-expressing cells established from grafted tumor fragments gave rise to tumors in immunocompetent mice. We have now compared gene expression profiles of primary, vGPCR-induced tumors, cell lines established from these tumors as well as the parenteral BALB/c-3T3 and vGPCR-transformed BALB/c-3T3 cells. This approach, in combination with ChIP-on-Chip histone modification analyses, large-scale DNA methylation profiling using Reduced-Representation Bisulfite Sequencing (RRBS), and mass spectrometry-based SILAC proteomics led to the identification of critical gene networks comprising signaling and immunomodulatory genes,

which are regulated by epigenetic modifications related to the successive passage of the vGPCR expressing 3T3 cells in nude and immunocompetent mice. Hence, this novel animal model will contribute to our understanding of the role of the tumor microenvironment for the induction of chromatin remodeling and epigenetic changes, which in turn trigger immune escape and progressive tumorigenesis.

Immunotherapy with bispecific and recombinant antibodies targeting Non-Hodgkin lymphoma and multiple lymphoma

(collaboration: G. Moldenhauer, DKFZ, Heidelberg)
Bispecific antibodies are promising agents for immunotherapy. We have developed a quadroma-based trifunctional bispecific antibody binding the chemokine receptor CXCR5 and the T cell antigen CD3 that efficiently prevents tumor growth in a mouse B cell lymphoma model. In vitro, the bispecific CXCR5::CD3 antibody efficiently recruited effector T cells to CXCR5 expressing B cells and induced a costimulation-independent activation of $CD8^+$ and $CD4^+$ T cells. In vivo imaging revealed that CXCR5::CD3 and its parental CXCR5 antibody efficiently prevent tumor growth in a xenograft model of B cell lymphoma in mice and prolonged their survival significantly. Our results identify CXCR5 as a promising target for antibody-based therapies in the treatment of B cell malignancies.

(collaboration: S. F. Marino, O. Daumke, MDC; J. Westermann, Charité)
Multiple myeloma (MM) is an aggressive incurable plasma cell malignancy. The lack of immunotherapies specifically targeting MM cells led us to develop a human-mouse chimeric antibody directed against an extracellular domain of a receptor molecule, which is almost exclusively expressed on plasma cells and MM cells. The high affinity antibody blocks the binding of the native ligand to this receptor. This finding was rationalized by the high resolution crystal structure of the Fab fragment in complex with the extracellular domain. Most importantly, the antibody effectively depleted MM cells *in vitro* and *in vivo,* substantially prolongs tumor-free survival under therapeutic conditions in a xenograft mouse model, and is therefore a promising option for the effective treatment of MM and autoimmune diseases.

Patents / Patent applications

An antibody that binds a plasma cell-specific antigen suitable for use in the treatment of plasma cell diseases such as multiple myeloma and autoimmune diseases (filing date 01.11. 2012).

Selected Publications

Vaeth M, Müller G, Stauss D, Dietz L, Klein-Hessling S, Serfling E, Lipp M, Berberich I, Berberich-Siebelt F (2014) Follicular regulatory T cells control humoral autoimmunity via NFAT2-regulated CXCR5 expression J Exp Med. 211:545-561. [Epub 2014 Mar 3]

Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Pillars in immunology article: two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999. 401:708-712 (2014) J Immunol. 192:840-844.

Baddack U, Hartmann S, Bang H, Grobe J, Loddenkemper C, Lipp M, Müller G (2013) A chronic model of arthritis supported by a strain-specific periarticular lymph node in BALB/c mice. Nat Commun. 4:1644. doi: 10.1038/ncomms2625.

Wichner K, Fischer A, Winter S, Tetzlaff S, Heimesaat MM, Bereswill S, Rehm A, Lipp M, Höpken UE (2013) Transition from an autoimmune-prone state to fatal autoimmune disease in CCR7 and RORγt double-deficient mice is dependent on gut microbiota. J Autoimmun. 47:58-72. [Epub 2013 Sep 24]

Schumann M, Winter S, Wichner K, May C, Kühl AA, Batra A, Siegmund B, Zeitz M, Schulzke JD, Lipp M, Höpken UE (2012) CCR7 deficiency causes diarrhea associated with altered ion transport in colonocytes in the absence of overt colitis. Mucosal Immunol. 5:377-387. [Epub 2012 Mar 7]

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Photo: David Ausserhofer/MDC

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Molecular Immunotherapy

Goal of our work is the implementation of basic research into preclinical models and clinical trials. Our strategies cover passive and active immunotherapy. A pilot clinical study with a gene-modified tumor cell vaccine developed in cooperation with Th. Blankenstein (MDC) and D. Schendel (GSF Munich) for treatment of advanced metastatic renal cancer has been concluded demonstrating feasibility and safety. We would like to combine this vaccine with antibodies targeting the PD1/PD-L1 pathway in patients who have residual disease after therapy with tyrosine kinase inhibitors such as sorafenib and sunitinib.
A multicenter clinical trial of Dendritic cell vaccination in chronic myeloid leukemia in patients with persisting minimal residual disease after imatinib treatment is due to start in early 2014. Preclinical models evaluate the costimulatory role of cytokines and chemokines such as CCL19 and CCL21 in DNA vaccination. This strategy is used to target Her-2, an antigen overexpressed in breast cancer where we plan a clinical study using a gene-gun delivery system. T-cell receptor (TCR) gene transfer is a promising strategy for adoptive immunotherapy of Lymphomas and Leukemias. Epitopes of the B-cell antigens CD22 and CD79a/b are being evaluated as targets in cooperation with Th. Blankenstein. We also evaluate the possibility of targeting hematopoietic lineage specific antigens in the context of allogeneic stem cell transplantation.

Clinical vaccination studies using dendritic cells or modified tumor cells for the induction of tumor specific immune responses

Jörg Westermann and J. Kopp, in cooperation with Th. Blankenstein and W. Uckert

In renal carcinoma (RCC), a gene-modified, immunogenic tumor cell vaccine that expresses co-stimulatory molecules and secretes Interleukin-7, generated in cooperation with Th. Blankenstein and W. Uckert (MDC) as well as D. Schendel (GSF, Munich) has been produced according to GMP rules in our laboratory at the Robert-Rössle Building. The pilot trial has been safely concluded. Evaluation of the immune response of vaccinated patients, and our recent data concerning the numbers of regulatory T cells in RCC patients undergoing therapy with the multikinase inhibitors sorafenib and sunitinib have led us to design a follow-up trial in conjunction with antibodies that increase T-cell stimulation by reducing regulatory T-cells.

Following on a successfully concluded clinical vaccination trial using *in vitro*-generated, bcr-abl positive dendritic cells (DC) in patients with chronic myeloid leukemia (CML) we are going to start a multicenter DC-vaccination clinical trial in CML patients with persisting minimal residual disease upon treatment with tyrosine-kinase inhibitors in 2014 with the aim of eradicating residual leukemia cells.

DNA Vaccination: preclinical models

Jörg Westermann, Tam Nguyen-Hoai in cooperation with U. Höpken and M. Lipp

DNA vaccination offers several advantages over the use of peptides as vaccines (DNA covers several MHC-I and MHC-II epitopes, directly targets the endogenous presentation pathway and contains immunostimulatory CpG sequences). Furthermore, DNA vaccines

can be easily produced on large-scale for the use in clinical trials across HLA barriers. In cooperation with the group of M. Lipp (Molecular Tumor Genetics) we have explored the possibility of recruiting immune cells by using plasmid DNA coding for the chemokines CCL19 and CCL21 as possible adjuvants. Coexpression of CCL19 or CCL21 with tumor antigens results in enhancement of a Th1-polarized immune response with substantial improvement of the protective effect of the vaccine. Further improvement of the vaccine potency has been achieved using a gene-gun for intradermal vaccine application (Cooperation with O.Hohn/S. Norley, the Robert-Koch Institute). These encouraging results have been extended to a preclinical model using the human breast-cancer associated antigen Her-2/neu as a target antigen, with the aim of developing a vaccine for breast cancer patients.

Adoptive T-cell therapy for lymphoma and leukemia

Simone Reuß, Sara Boiani & Neşe Çakmak in cooperation with Th. Blankenstein and W. Uckert

We are focusing on the treatment of hematological malignancies, particularly of B cell origin. Addressing possible therapies, we are working on the development of T cell receptor gene therapy. The retroviral transfer of TCR genes into donor T lymphocytes equips them with a new specificity which can be directed against antigens presented by the malignant cells. Upon transfer into patients, the gene-modified T cells are able to attack and kill antigen-expressing cells. As a target antigen expressed by most B cell-caused hematological disorders we have chosen the receptor CD22 which is responsible for B cell interaction and regulation of B cell signaling. Besides CD22, we are interested in a second B cell antigen for targeted immunotherapy. CD79 is a heterodimeric receptor which is associated with the B cell receptor complex and regulates its signaling. It is expressed on almost all B cell neoplasms. So far, these antigens (CD22 and CD79a/b) have been targeted mainly by antibodies but not by T cell therapy. In cooperation with Th. Blankenstein (MDC), we make use of a humanized mouse model expressing the human TCR gene repertoire to identify CD22 or CD79a/b-specific T cells which have not been thymically selected for the human CD22 or CD79a/b proteins in the murine system and are therefore of high affinity. Currently, we are investigating if CD22 and CD79a/b are suitable targets for TCR gene therapy by analyzing their potential epitopes and testing specific TCRs for gene therapy. In the context of a cooperative project we also evaluate mutation-specific epitopes of key regulators of lymphocyte pathways such as MYD88, CARD11, EZH2, A20, CD79a and CD79b as possible target for TCR recognition. Several hematopoiesis lineage specific antigens are also being evaluated as targets in the context of allogeneic stem cell transplantation.

Patents / Patent applications

PCT-Anmeldung PCT/EP2013/069569 "CD22-BINDING PEPTIDES" (O. Schmetzer and A. Pezzutto)

Selected Publications

Scholz C, Pinto A, Linkesch W, Linden O, Viardot A, Keller U, Hess G, Lastoria S, Lerch K, Frigeri F, Arcamone M, Stroux A, Frericks B, Pott C and Pezzutto A. 90Yttrium-Ibritumomab-Tiuxetan as First Line Treatemnt for Follicular Lymphoma: 30 months of follow-up Data from an International Multicetner Phase II Clinical Trial. (2013) J Clin Oncol 31: 308-313

Nguyen-Hoai T, Baldenhofer G, Ahmed MS, Pham-Duc M, Gries M, Lipp M, Dörken B, **Pezzutto A**, Westermann J. CCL19 (ELC) improves TH1-polarized immune responses and protective immunity in a murine Her2/neu DNS vaccination model. J Gene Med. 2012 14:128-137

Nguyen-Hoai, T., Baldenhofer, G., Sayed Ahmed, M.S., Pham-Duc, M., Vu, M.D., Lipp, M., Doerken, B., **Pezzutto, A.**, and Westermann, J. (2012). CCL21 (SLC) improves tumor protection by a DNA vaccine in a Her2/neu mouse tumor model. Cancer Gene Therapy 19: 69-76.

Westermann, J., Florcken, A., Willimsky, G., van Lessen, A., Kopp, J., Takvorian, A., Johrens, K., Lukowsky, A., Schonemann, C., Sawitzki, B., Pohla, H., Frank, R., Dorken, B., Schendel, D.J., Blankenstein, T., and **Pezzutto, A.** (2011). Allogeneic gene-modified tumor cells (RCC-26/IL-7/CD80) as a vaccine in patients with metastatic renal cell cancer: a clinical phase-I study. Gene Therapy 18, 354-363.

Buchner A, Pohla H, Willimsky G, Frankenberger B, Frank R, Baur-Melnyk A, Siebels M, Stief CG, Hofstetter A, Kopp J, **Pezzutto A**, Blankenstein T, Oberneder R, Schendel DJ. (2010). Phase I trial of allogeneic gene-modified tumor cell vaccine RCC-26/CD80/IL-2 in patients with metastatic renal cell carcinoma. Hum Gene Ther. 21: 285-297.

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Immune Regulation and Cancer

Having moved from Harvard Medical School to the MDC towards the end of 2011, our group has continued its exploration of signals, transcriptional networks and post-transcriptional regulators like miRNAs controlling cell-autonomous and cell interaction-based mechanisms of normal and malignant B cell development, using conditional targeted mutagenesis in mice as our main experimental tool. Main achievements have been the elucidation of the role of c-Myc in germinal center (GC) B cell differentiation and the generation of faithful pre-clinical models of certain classes of human B cell lymphomas and Epstein-Barr-Virus (EBV) pathologies, including the immune surveillance of EBV-infected normal and transformed cells. New, highly efficient systems of targeted mutagenesis in mammalian cells, in particular CRISPR/Cas9-mediated mutagenesis, are presently revolutionizing the fields of mammalian somatic cell genetics and the generation of genetically modified experimental animals. Accordingly, a main recent activity of the group has been the introduction of this new approach into our experimental routine.

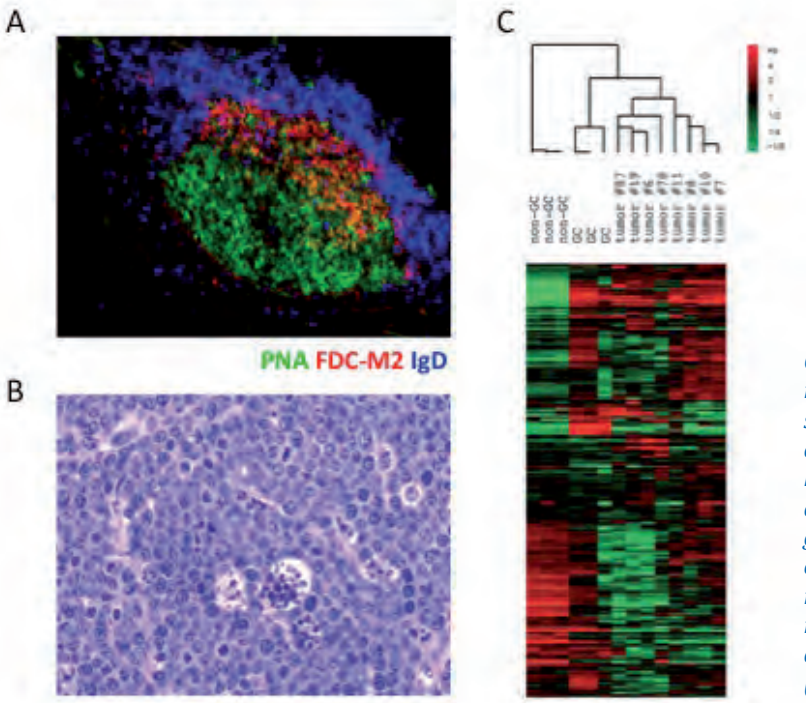
Mechanisms controlling B cell development and homeostasis

Dinis Calado, Emmanuel Derudder, Robin Graf, Verena Labi, Sandrine Sander

While our previous work in this area had focused on survival and activation signals emanating from the B cell antigen receptor (BCR) and the BAFF receptor (BAFFR) and identified PI3K signaling as a key survival signal in resting mature B cells downstream of the BCR, apart from "alternative" NF-κB signals downstream of BAFFR and canonical NF-κB signaling upon B cell activation through the BCR and Toll-like receptors (TLRs) & CD40, a main effort over the last years has been to clarify the role of the proto-oncogene c-Myc in the GC reaction. The GC reaction is a key process in T cell dependent antibody responses, where antigen-activated B cells proliferate vigorously while undergoing programmed DNA breaks accompanying somatic hypermutation (SHM) of antibody variable (V) region genes and antibody class switch recombination (CSR).

Earlier work had suggested that GC B cell proliferation might be independent of c-Myc, driven perhaps by a program controlled by the transcription factor Bcl6. Using conditional gain- and loss-of-function alleles of c-Myc we showed that c-Myc is critical at two distinct stages of the GC response, namely its initiation and when somatic antibody mutants arising in the course of the reaction are selected for further rounds of cell division & somatic mutation on the basis of their affinity to antigen(Calado et al. 2012). This result provides an explanation for the frequent (transcription-dependent) c-Myc translocations in GC B cell–derived B cell lymphomas, by far the most frequent class of such tumors.

Modeling Burkit lymphoma, a GC-B cell-derived human malignandy, in mice



GC B cells (= PNA expressing cells (A)) are important for the generation of antigen-specific immune responses; additionally, they are the cellular origin of most B cell derived lymphomas, like e.g. BL. We have generated a pre-clinical model of BL by conditional targeted mutagenesis allowing the expression of oncogenic factors specifically in GC B cells that resemble the human disease in several aspects including histology ("starry sky" pattern (B)) and expression of GC B cell specific genes (C) (Sander et al. 2012).

Other experiments on this subject have addressed the role of BCR signaling in B cell subset differentiation, substantiating our previous finding that mature follicular B cells, the major subset of peripheral B cells, can differentiate into cells of the so-called B1 subset upon exchange of their BCR by a BCR typical for B1 cells. Present work addresses the mechanism of this "transdifferentiation" process, which proceeds through an initial phase of rapid proliferation, and its relation to B1 cell development in ontogeny.

We have also continued studies on the role of miRNAs in hematopoietic development, with a focus on B cell differentiation and miRNA-142 and the miRNA cluster miR-17~92. This work relies heavily on attempts to identify miRNA targets at a genome-wide level (collaborating with colleagues at the BIMSB), and conditional mutagenesis of miRNA seed matches in the 3' UTRs of presumed target genes. A surprising cell type specificity of miRNA control is beginning to emerge from these experiments. The work also relates to the impact of miRNA control on the pathogenesis of lymphomas (Jin et al. 2013).

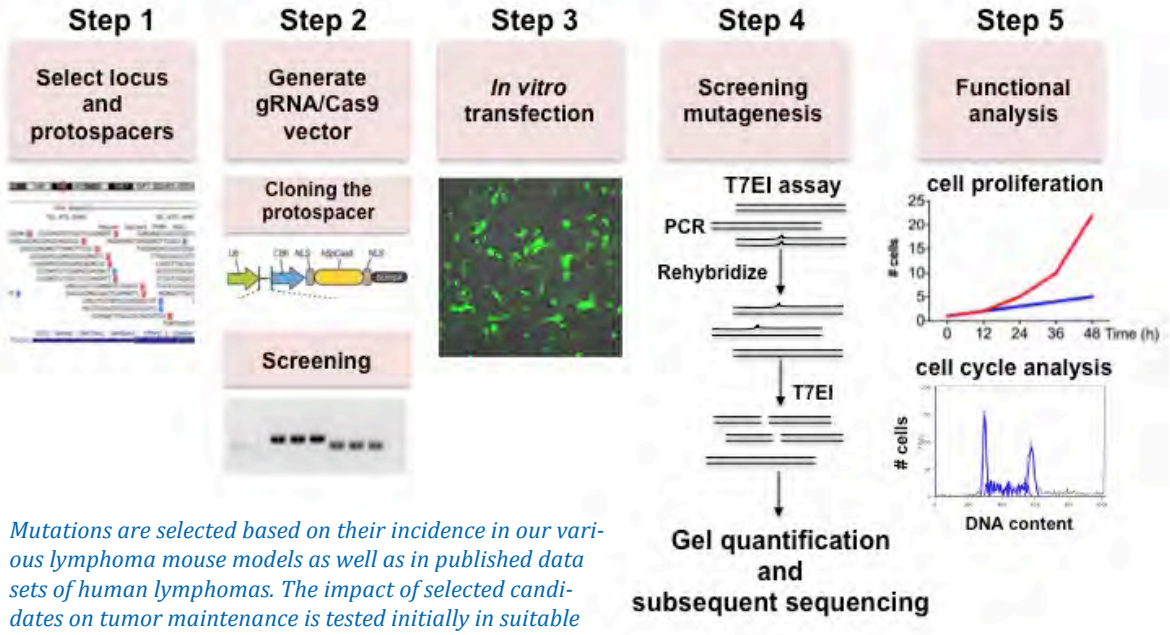
Modeling human lymphomas and EBV-driven pathologies and immune surveillance in mice

Burkitt lymphoma

Sandrine Sander, Daniela Bolgehn, Van Trung Chu, Karl Koechert, Dinis Calado

Burkitt lymphoma (BL) is a GC-derived human B cell lymphoma, known to be driven in 100% of the cases by deregulated c-Myc expression. The latter is mostly mediated by cMyc translocations into the immunoglobulin (Ig) loci of the cells. As these translocations involve the non-productively rearranged Ig loci and thus keep BCR expression by the cells intact, we speculated that the pro-survival signal required to neutralize c-Myc's pro-apoptotic properties would be the PI3K survival signal downstream of the BCR, which we had previously identified. Indeed, combining conditional constitutive c-Myc expression and PI3K signaling in GC B cells by conditional targeted mutagenesis in mice resulted in a faithful preclinical model of BL in terms of localization, histological & cell surface phenotype and gene expression signature. The tumors also shared recurrent tertiary somatic mutations

Analysis of tumor associated mutations by CRISPR/Cas9



Mutations are selected based on their incidence in our various lymphoma mouse models as well as in published data sets of human lymphomas. The impact of selected candidates on tumor maintenance is tested initially in suitable (mouse and human) cell lines according to the indicated workflow of CRISPR/Cas9 mediated mutagenesis.

with their human counterparts (Sander et al. 2012). Significantly, the mouse tumors exhibited the same tendency to disseminate into the central nervous system as human BL, the most threatening clinical complication of the disease. Ongoing experiments address this issue and use CRISPR/Cas9-based mutagenesis to address the impact of recurrent somatic mutations on BL pathogenesis.

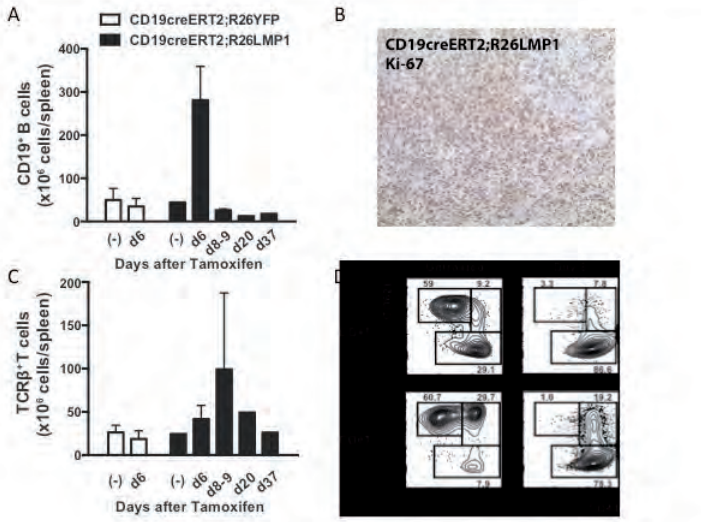
EBV-driven pathologies and immune surveillance; Hodgkin Lymphoma (collaboration with the Janz/Mathas/Dörken group)

Tomoharu Yasuda, Shuang Li, Thomas Sommermann, Timm Weber, Tristan Wirtz

EBV is a γ herpes virus endemic in the human population, with over 90% of people infected worldwide. The virus infects and transforms human B cells, but is contained by powerful T and NK cell immunosurveillance. In infected individuals EBV persists in a few B cells for life. Reactivation under conditions of immune suppression like in post-transplantation or AIDS patients can lead to a life-threatening lymphoproliferation and EBV-driven B cell lymphomas. These complications also occur upon primary EBV infection, in rare patients which are genetically unable to control EBV. In X-linked Lymphoproliferative Syndrome (XLP), T and NK cell defense against EBV is compromised by mutation of the X-linked gene encoding the signal-transducer SAP. EBV-driven lymphomas also occur in other contexts. A case of special interest is Hodgkin Lymphoma (HL), where the tumor cells have undergone a clonal EBV infection in about 40% of the cases.

Starting from our discovery many years ago that the tumor cells in HL, the so-called Hodgkin and Reed-Sternberg (HRS) cells, arise from pro-apoptotic ("crippled") GC B cells, we have over the years attempted to model HL and EBV infection in the mouse. With respect to HL pathogenesis, this includes the conditional activation of HL-associated transcriptional regulators and survival factors in mouse B cells, particularly at the GC stage of differentiation, a project that is carried out in cooperation with the Janz/Mathas/Dörken group. Our attempts to model EBV infection are based on the transgenic expression of key EBV proteins in the B cell lineage of mice, a species from which EBV is absent. Focusing on the EBV proteins LMP1 and LMP2A, known to play key roles in the transformation of human B cells, we showed in work which was already initiated in Boston that LMP-expressing (mouse) B cell blasts induce efficient immune surveillance in mice, and that LMP-driven lymphomas rapidly develop when immune surveillance is compromised (Zhang, Kracker, Yasuda, Casola et al. 2012). More recent work has shown that we can faithfully model primary EBV infection in mice by conditional activation of LMP expression in a small fraction of mouse B cells at various stages of differentiation (Yasuda et al. 2013).

A mouse model of primary EBV infection in mice



Mouse model for primary EBV infection. (A) Tamoxifen-induced expression of LMP1 in mouse B cells leads to expansion of splenic B cells in experimental (CD19creERT2;R26LMP1), but not control mice (CD19creERT2;R26YFP). (B) Proliferating lymphocytes six days after tamoxifen-treatment of a CD19creERT2;R26LMP1 mouse. Ki-67 staining of proliferating cells in a spleen section. (C) T cell expansion after LMP1+ B cell expansion in spleen. (D) An effector/memory phenotype of T cells (CD44+/CD62L-) following expansion (Yasuda et al. 2013).

In this model, an initial rapid expansion of LMP-expressing B cells is followed by the activation and expansion of T cells, with subsequent elimination of the B cell blasts and establishment of T cell memory. Interference with T cell defense results in fatal lymphoproliferation as in human XLP. Ongoing work addresses the mechanisms controlling immune surveillance in this system, which apparently reflects a strategy of the virus to establish a profitable coexistence with its (human) host, to model human XLP and EBV-driven lymphomagenesis in preclinical mouse models, and to elucidate cell-autonomous and cell interaction-based mechanisms driving EBV-associated pathologies.

Other related work

Dinis Calado, Ulrike Sack, Kristin Schmidt, Van Trung Chu, Baochun Zhang; collaboration with Wiebke Winkler and Martin Janz

In work that has been submitted for publications, we have shown that in addition to the well-known role of canonical NF-κB signaling in the pathogenesis of Diffuse Large B Cell Lymphoma of the Activated B cell-type (ABC-DLBCL), the alternative NF-κB pathway can also functionally contribute to DLBCL pathogenesis, in concert with other genetic alterations. We are also in the process of functionally elucidating the role of mutations in the TLR and BCR signaling pathways in the pathogenesis of DLBCL, through targeted conditional mutagenesis in mice. Finally, ongoing work in collaboration with Wiebke Winkler and Martin Janz addresses the generation of new mouse models of human multiple myeloma.

Selected Publications

Yasuda T, Wirtz T, Zhang B, Wunderlich T, Schmidt-Supprian M, Sommermann T, Rajewsky K. Studying Epstein-Barr Virus Pathologies and Immune Surveillance by Reconstructing EBV Infection in Mice. Cold Spring Harbor Symposia on Quantitative Biology. 2013 Nov 15.

Jin HY, Oda H, Lai M, Skalsky RL, Bethel K, Shepherd J, Kang SG, Liu W-H, Sabouri-Ghomi M, Cullen BR, Rajewsky K, Xiao C. MicroRNA-17~92 plays a causative role in lymphomagenesis by coordinating multiple oncogenic pathways. EMBO J. 2013 Aug;32(17):2377–91.

Calado DP, Sasaki Y, Godinho SA, Pellerin A, Köchert K, Sleckman BP, de Alboran IM, Janz M, Rodig S, Rajewsky K. The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. Nat Immunol. 2012 Nov;13(11):1092–100.

Sander S, Calado DP, Srinivasan L, Köchert K, Zhang B, Rosolowski M, Rodig SJ, Holzmann K, Stilgenbauer S, Siebert R, Bullinger L, Rajewsky K. Synergy between PI3K Signaling and MYC in Burkitt Lymphomagenesis. Cancer Cell. 2012 Aug 14;22(2):167–79.

Zhang B, Kracker S, Yasuda T, Casola S, Vanneman M, Hömig-Hölzel C, Wang Z, Derudder E, Li S, Chakraborty T, Cotter SE, Koyama S, Currie T, Freeman GJ, Kutok JL, Rodig SJ, Dranoff G, Rajewsky K. Immune surveillance and therapy of lymphomas driven by Epstein-Barr virus protein LMP1 in a mouse model. Cell. 2012 Feb 17;148(4):739–51.

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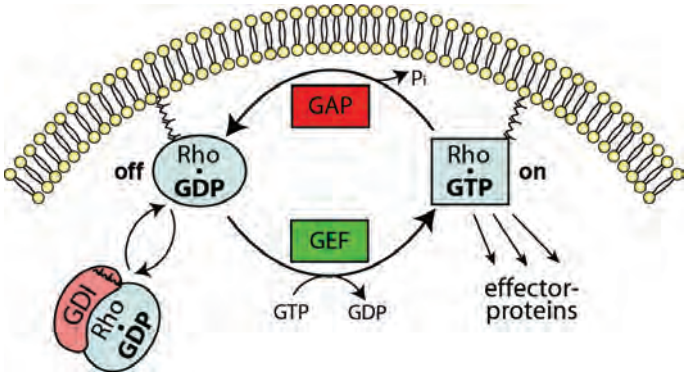
Oliver Rocks

Spatio-temporal Control of Rho GTPase Signaling

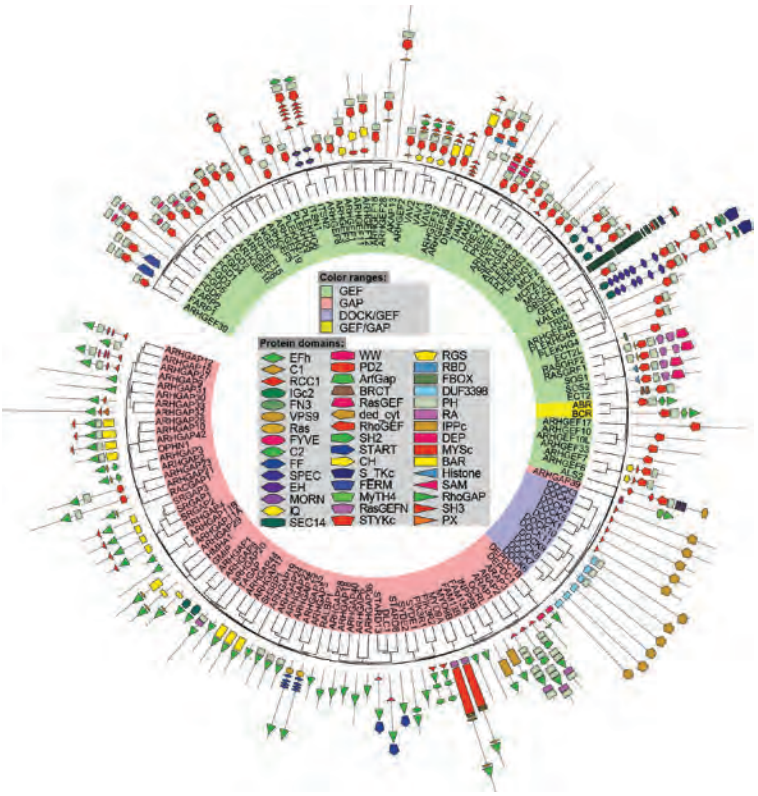
Cells frequently change their shape and their attachment to the environment in order to move or to adopt specialized structures in tissues. These events are fundamental during embryonic development, immune surveillance or wound repair. The reorganization of the cytoskeleton and the adhesion machinery thereby is highly dynamic and requires precise coordination. Any deregulation can contribute to cancer metastasis and various developmental, cardiovascular and neurological disorders. Our lab is interested in the family of Rho GTPase proteins which have emerged as master regulators of the cytoskeleton. We combine advanced microscopy with cell biological and genetic approaches to study the precision control that ensures proper Rho signaling dependent cellular responses. Specificity of Rho GTPase function is achieved by the huge family of RhoGEF and GAP regulatory proteins. We aim to identify mechanisms by which these proteins couple Rho activity to specific environmental cues and functional contexts in a cell. Insights into this spatio-temporal regulation will provide a deeper understanding of the processes that drive specific cell morphology changes, in normal and disease settings.

Signaling Specificity of Rho GTPases

Rho GTPases are molecular switches that cycle between an 'on' and 'off' state. Only in the activated state they bind effector proteins and thereby relay incoming signals further downstream of a signaling pathway. This cycle is controlled by three classes of regulatory proteins: the large families of activating RhoGEFs (Rho guanine nucleotide exchange factors) and inactivating RhoGAPs (Rho GTPase activating proteins), and by RhoGDI proteins (Rho guanine nucleotide dissociation inhibitors), which mediate the sequestration of the membrane anchored Rho GTPases into the cytosol (Fig. 1). The Rho family comprises about 20 genes, with the most prominent members RhoA, Rac1 and Cdc42. Numerous Rho-regulated signaling pathways have been outlined over the past years, many of which drive central aspects of cellular morphodynamics. The different members of the Rho family thereby have distinct functions and can crosstalk with each other, resulting in a complex interplay of pathways. A precise spatio-temporal control of their activities is essential to assure specificity in the biological response. The major challenge in the field thus is to understand how this signaling specificity is achieved by the RhoGEF/GAP/GDI regulatory proteins. Notably, deregulated Rho GTPase signaling in pathological settings is mostly caused by alterations at the level of



Schematic representation of the molecular switch function of Rho GTPases and the function of their regulatory proteins.



Modular domain architecture of the human RhoGEF and RhoGAP proteins.

the regulatory proteins and not by mutations of the Rho proteins themselves.

RhoGEFs and RhoGAPs

The genome encodes over 145 different RhoGEFs and GAPs which contain a diverse spectrum of subcellular targeting and protein interaction domains, besides their catalytic domains (Fig. 2). This multidomain architecture enables them to assemble specific protein complexes to target Rho activity to distinct signaling contexts such as focal adhesions, cell junctions, endocytic vesicles or mitotic spindles. However, the majority of RhoGEFs and GAPs is only poorly characterized and the cellular repertoire of spatio-temporal control of Rho GTPases not fully explored. Therefore, we have recently carried out a systematic initial characterization of all mammalian RhoGEFs and GAPs, yielding a complete cDNA library of all regulators and datasets of their interactome, subcellular localization, overexpression and knockdown phenotypes. We now exploit this unique resource and study novel RhoGEFs and GAPs with implications in different signaling contexts such as cell adhesion, guided cell migration or angiogenesis. To this end, we use live cell microscopy and biosensors to obtain information on appropriate spatial and temporal microscales and employ three-dimensional cell culture systems and model organisms that closely reflect the underlying biological processes in the organismal context. We aim for a broader understanding of the function of the context-specific signaling scaffolds, which essential components they contain, how their assembly is regulated, how they relay signals to the cytoskeleton or are interlinked with it and how they communicate with other cellular signaling pathways.

Temporal framework of Rho GTPase signaling

Very little is known also about the time control of Rho signaling. Most Rho family GTPases are lipid modified and need to be membrane associated to properly exert their signaling function. Exactly how on membranes the formation of Rho signaling complexes is initiated, maintained and terminated and whether a precise temporal control is important for proper signaling is unclear and is technically difficult to be studied in cells. We employ state-of-the-art imaging techniques to analyze the dynamics of membrane interaction of Rho GTPases and its modulation by regulatory and effector proteins. We thereby aim to elucidate the temporal framework in which Rho signaling complexes operate and to find novel modes of signal modulation.

Selected Publications

Rocks, O., Gerauer, M., Vartak, N., Koch, S., Huang, ZP., Pechlivanis, M., Kuhlmann, J., Brunsveld, L., Chandra, A., Ellinger, B., Waldmann, H., Bastiaens, PI. (2010) The Palmitoylation Machinery Is a Spatially Organizing System for Peripheral Membrane Proteins. **Cell** 141(3):458-471

Dekker, FJ.*, **Rocks, O.***, Vartak*, N., Menninger*, S., Hedberg, C., Balamurugan, R., Wetzel, S., Renner, S., Gerauer, M., Schölermann, B., Rusch, M., Kramer, JW., Rauh, D., Coates, GW., Brunsveld, L., Bastiaens, PI., Waldmann, H. (2010) Small-molecule inhibition of APT1 affects Ras localization and signaling. **Nat Chem Biol** 6(6):449-56 *equal contribution

Rocks, O., Peyker, A. & Bastiaens, PI. (2006) Spatio-temporal segregation of Ras signals: one ship, three anchors, many harbors. **Curr Op Cell Biol** 18(4):351-7

Rocks, O., Peyker, A., Kahms, M., Verveer, PJ., Koerner, C., Lumbierres, M., Kuhlmann, J., Waldmann, H., Wittinghofer, A. & Bastiaens, PI. (2005) An Acylation Cycle Regulates Localization and Activity of Palmitoylated Ras Isoforms. **Science** 307:1746-1752

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Photo: David Ausserhofer/MDC

Claus Scheidereit

Signal Transduction in Tumor Cells

The major interest of our laboratory is to understand gene expression and signal transduction processes in tumorigenesis and in development. The inducible transcription factor nuclear factor-κB (NF-κB) provides an excellent model system with broad physiological and medical significance. The NF-κB system controls important steps in immunity and inflammation, as well as cell death, proliferation or metabolism. We have investigated various aspects of the regulation of NF-κB transcription factors, whose activities are controlled by inhibitory IκB proteins and the IκB kinase (IKK) complex. Our major efforts are to decipher the mechanisms that underlie gene regulation by IKK and NF-κB in development and in the pathogenesis of human diseases.

The molecular constituents of IKK and NF-κB signaling cascades

The mammalian NF-κB family comprises five related members, p50, p52, p65, c-Rel and RelB, which form hetero- and homodimers and regulate hundreds of genes in the context of multiple important physiological and pathological processes. The inhibitory IκB proteins, e.g. the cytoplasmic IκBα or the nuclear Bcl-3 and IκB-ζ, are fundamental regulators NF-κB activity. Two NF-κB subunits, p50 and p52, are generated by proteasomal processing of their precursor proteins, p105 and p100, respectively.

NF-κB activation requires the IκB kinase (IKK) complex, composed of the two kinases (IKKα and IKKβ) and the regulatory subunit IKKγ (also known as NEMO). The IKK complex responds to a wide variety of extra- and intracellular stimuli to catalyze phosphorylation of IκB and NF-κB proteins as well as of other substrates. A characteristic feature of canonical signaling cascades is the modification of TRAF or RIP proteins with K63-linked or linear polyubiquitin chains, which mediates recruitment of signaling components with ubiquitin binding domains, including IKKγ, finally resulting in IKK activation. Catalytically active IKK complexes trigger degradation of IκBs and liberation of prototypic p50-p65. The non-canonical NF-κB pathway is activated by a specific set of receptors and induces C-terminal processing of NF-κB2/p100 to p52 and nuclear translocation of p52-RelB complexes. The non-canonical pathway proceeds with much slower kinetics and requires NF-κB-inducing kinase (NIK) and IKKα. A third IKK pathway is activated by DNA double strand breaks and depends on nuclear shuttling and SUMO-modification of IKKγ, as well as on the kinase ATM.

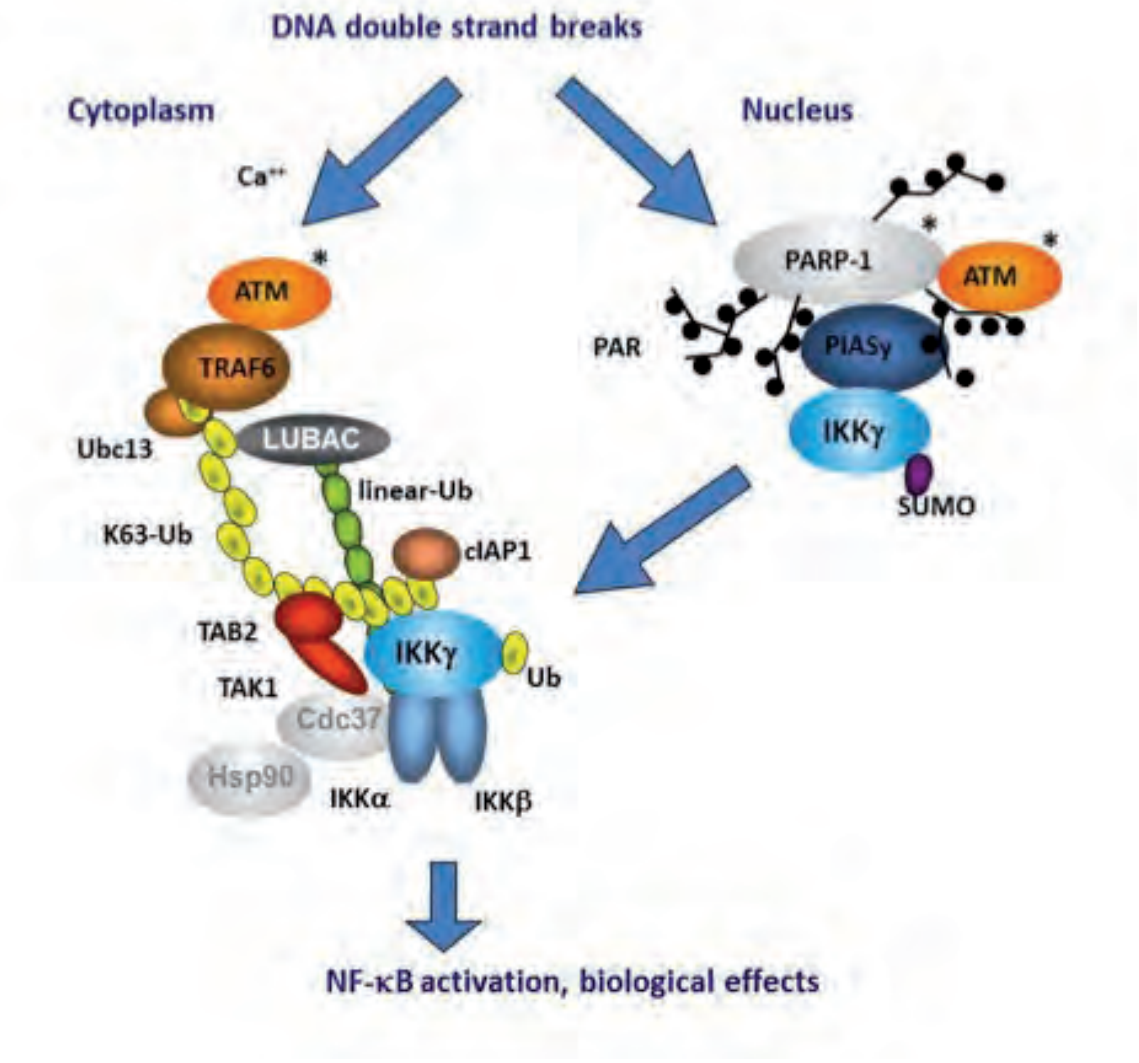


Figure 1: *DNA damage invokes two signaling axes, triggered by ATM and PARP-1, to activate NF-κB. PARP-1 is activated and sequesters PIASy, ATM and IKKγ, which shuttles between nucleus and cytoplasm, resulting in IKKγ SUMOylation. ATM is exported to the cytoplasm and binds to TRAF6 to induce Ubc13 dependent K63-linked polyUb synthesis. TRAF6 ubiquitination activates TAK1 and cIAP1-dependent IKKγ monoubiquitination. IKKγ-monoUb requires both, PARP-1 and ATM signals and is essential for IKK activation. Other components acting downstream of TRAF6 and IKK, respectively, are the linear ubiquitin chain assembly complex (LUBAC) and the Hsp90/Cdc37 chaperone complex.*

Mechanism of IKK and NF-kB activation by genotoxic stress

(Seda Çöl Arslan, Patrick Beaudette, Daniel Heinze, Michael Hinz, Marina Kolesnichenko, Nadine Mikuda, Giulietta Roel, Michael Stilmann, Michael Willenbrock)

Metabolic and external DNA-damaging agents, such as ionizing radiation or toxic chemicals, constantly endanger the genomic integrity of cells. To counter these threats, mammalian cells have developed a network of DNA damage response. DNA lesions are recognized by sensor proteins, which initiate DNA repair, cell cycle checkpoint control and the activation of transcription factors such as NF-κB and p53. It is generally assumed that p53 induces cell-cycle arrest or apoptosis, while NF-κB triggers cell survival and may counteract apoptotic action of genotoxic therapy. However, it could be demonstrated that NF-κB activation by chemotherapy can also be beneficial through the induction of a senescence-associated secretory phenotype (Jing et al. 2011). We could demonstrate that genotoxic stress triggers two corresponding signaling axis to activate the IκB kinase (IKK) complex

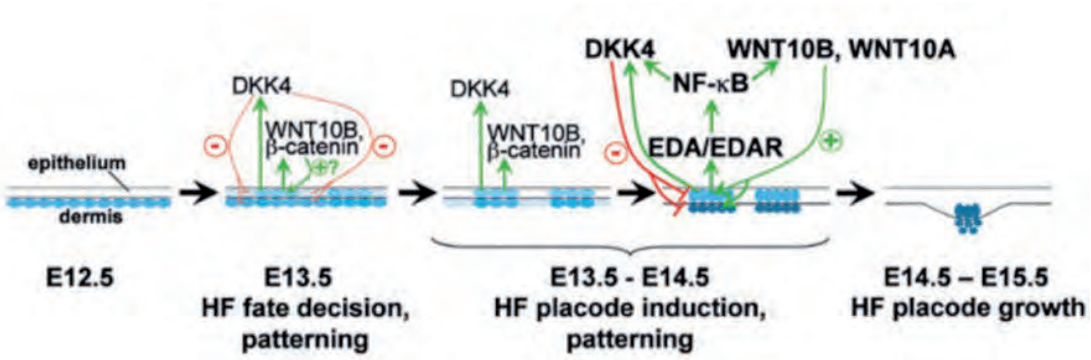


Figure 2: *Molecular controls of early hair follicle development in the mouse (see Zhang et al., 2009, for details). At embryonic day E12.5, WNT is uniformly active in the upper dermis. First focal epidermal and dermal WNT activity was observed at E13.5, and is entirely dependent on epidermal WNT/β-catenin activity. The only focally expressed WNT at E13.5 is WNT10b, suggesting that it may be the HF placode inducing WNT. Early WNT10b activity initiates expression of the WNT inhibitor Dkk4, which may prevent WNT signaling in the direct surroundings of the developing placode. Between E13.5 and E14.5 Edar expression is focally up-regulated by WNT/β-catenin. This leads to activation of EDAA1/EDAR/NF-κB signaling, which induces expression of NF-κB target genes, such as Dkk4, Wnt10b and Wnt10a. Higher concentrations of DKK4 protein caused by joint NF-κB and β-catenin transcriptional activity will further refine hair follicle placode patterning. Apart from Dkk4, EDA-A1/EDAR/NF-κB signaling also maintains expression of Wnt10b, and perhaps also of WNT10a and Lef-1, leading to a positive feedback loop between WNT and EDA-A1 signaling.*

analogously to the canonical NF-κB signaling cascades (Figure 1). The first axis is initiated by the DNA strand break sensor poly(ADP-ribose)-polymerase-1 (PARP-1), which assembles a transient nucleoplasmic complex and triggers PIASy mediated SUMOylation and most likely ataxia telangiectasia mutated (ATM) mediated phosphorylation of nuclear IKKγ (Stilmann et al., 2009). It is assumed that modified IKKγ shuttles back to the nucleus and integrates into IKK complexes. ATM translocates to cytoplasm, binds to TRAF6 and triggers its K63-linked polyubiquitination. Activated TRAF6 recruits cIAP1 and TAB2-TAK1, resulting in TAK1 activation and IKKβ phosphorylation (Hinz et al., 2010). The final activation of the IKK complex requires IKKγ mono-ubiquitination of IKKγ at lysine 285, identified by "selected reaction monitoring" (SRM) mass spectrometry (MS) (collaboration with G. Dittmar). In ongoing studies, we investigate characteristics of the genotoxic pathway in vivo by bio-imaging, analyze how DNA damage induced IKKγ modifications contribute to NF-κB activation and search for novel components in the signaling cascade. Furthermore, we investigate the cross talk between p53 and NF-κB in transcriptional regulation.

Mechanism of NF-κB precursor processing and selective functions of canonical and non-canonical NF-κB/IKK gene networks

(Patrick Beaudette, Inbal Ipenberg, Eva Kärgel, Kívia A. Pontes de Oliveira, Linda von Hoff, Buket Yilmaz)

Hodgkin's lymphoma (HL), diffused large B-cell lymphoma (DLBCL) and many other tumor types rely on IKK and NF-κB to promote, amongst others, their proliferation and survival. In HL cells, canonical as well as non-canonical IKK/NF-κB signaling is constitutively activated. We investigate at a genome and transcriptome wide level how canonical (p50, p65), non-canonical (p52, IKKα, NIK) and accessory components (Bcl-3) of the NF-κB system control distinct tumor-biological functions and how gene specificity is reached (collaboration with N. Hübner and M. Andrade). The final aim is a full dissection of the IKK-dependent oncogenic network in HL cells in order to predict novel therapeutic targets. We have determined the complete genomic binding maps for canonical and non-canonical NF-κB species by ChIP sequencing and characterized their differential transcriptomic impact, using siRNA-mediated knockdown. In further

studies, we investigate the mechanisms and structures that underlie non-canonical NF-κB signaling, e.g. p100 to p52 conversion, using biochemical analyses, MS-based quantification of pathway components and mathematical modeling (collaboration with Gunnar Dittmar and Jana Wolf).

Role of Eda-A1/Edar/NF-κB signaling in development and organ regeneration

(Karsten Krieger, Philip Tomann und Ruth Schmidt-Ullrich)

To elucidate the in vivo function of NF-κB in embryonic development and organ regeneration, various loss-of-function, gain-of-function and reporter mouse models were generated in our laboratory. We previously demonstrated a key role for NF-κB in the induction of hair follicle and secondary lymph node development, and in the morphogenesis of ectodermal organs such as teeth or mammary glands. Further studies mainly concentrated on hair follicle development. Essential signals that regulate early hair follicle development include WNT/β-catenin, as well as TNF family member ectodysplasin A1 (EDA-A1) and its receptor EDAR. WNT/b-catenin signaling specifies the initial hair fate decision of epidermal keratinocytes, while NF-κB is required for hair bud formation. We have demonstrated an interdependence of WNT/β-catenin and EDA-A1/EDAR/NF-kB signaling which is required for focal hair bud formation and patterning, as well as hair bud down-growth (Zhang et al. 2009). To unravel further functions of NF-κB in ectodermal organogenesis, we have investigated NF-κB-dependent target gene signatures during hair follicle development. Our data indicate that NF-κB has a multifunctional role in hair follicle induction, including ECM remodeling and the regulation of stem cell markers. The function of NF-κB in stem cell biology and organ regeneration in hair follicles and in the intestine is currently under investigation.

Selected Publications

Stilmann, M., Hinz, M., Çöl Arslan, S., Zimmer, A., Schreiber, V.and Scheidereit, C. (2009) A nuclear poly(ADP-ribose) dependent signalosome confers DNA damage induced IkB kinase activation. **Mol Cell** 36, 365-378.

Zhang, Y., Tomann, P., Andl, T., Gallant, N., Huelsken, J., Jerchow, B., Birchmeier, W., Paus, R., Piccolo, S., Mikkola, M.L., Morrisey E.E., Overbeek, P.A:, Scheidereit, C., Millar, S.E., and Schmidt-Ullrich, R*. (2009) Reciprocal requirements for Eda/Edar/NF-κB and Wnt/β-catenin signaling pathways in hair follicle induction. **Dev. Cell** 17, 49-61.

Hinz, M.,Stilmann, M., Çöl Arslan, S., Khanna, K.K., Dittmar, G., and Scheidereit, C. (2010) A cytoplasmic ATM-TRAF6-cIAP1 module links nuclear DNA damage signaling to ubiquitin-mediated NF-κB activation. **Mol Cell,** 40, 63-74.

Jing H, Kase J, Dörr JR, Milanovic M, Lenze D, Grau M, Beuster G, Ji S, Reimann M, Lenz P, Hummel M, Dörken B, Lenz G, Scheidereit C, Schmitt CA, Lee S. (2011) Opposing roles of NF-κB in anti-cancer treatment outcome unveiled by cross-species investigations. **Genes Dev** 25, 2137-2146.

Nogai H, Wenzel SS, Hailfinger S, Grau M, Kaergel E, Seitz V, Wollert-Wulf B, Pfeifer M, Wolf A, Frick M, Dietze K, Madle H, Tzankov A, Hummel M, Dörken B, Scheidereit C, Janz M, Lenz P, Thome M, Lenz G. (2013) IκB-ζ controls the constitutive NF-κB target gene network and survival of ABC DLBCL. **Blood** 122, 2242-2250.

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Photo: private

Clemens A. Schmitt

Cancer Genetics and Cellular Stress Responses

Our scientific interests focus on cellular stress responses (so called 'failsafe mechanisms') that may serve as oncogene-evoked anti-tumor barriers. Ultimate stress responses such as apoptosis or cellular senescence – both terminal "cell-cycle exit" programs – do not only counter tumorigenesis, but are induced as effector mechanisms in response to anti-cancer chemotherapy as well. Importantly, many of the mutations now being increasingly unveiled by large-scale sequencing efforts of primary patient tumor biopsies throughout virtually all cancer entities might impair these cellular stress responses. However, the actual function of a distinct mutant gene product – determined by its integration in a complex signaling network – often remains elusive. Moreover, additional layers of information – namely the transcriptome, proteome and metabolome – are needed for a better understanding of the malignant blueprint, and, ultimately, to make the concept of "personalized medicine" a clinical reality. To test the impact of molecular lesions in cellular stress response programs on tumor development and treatment outcome under most physiological conditions in vivo, *we generate mouse models harboring lymphomas (and other tumor entities) with defined genetic defects, and study their biology, metabolism and long-term outcome* in vitro *and* in vivo.

Role of deregulated NF-κB and p53 signaling networks in lymphoma development and therapy

Soyoung Lee, Maurice Reimann, Julia Kase and collaboration partners (Claus Scheidereit, Jana Wolf, Gunnar Dittmar and others)

NF-κB and p53 signaling networks are intimately linked, since they regulate each other in complex ways. They partly cooperate in effector mechanisms (*i.e.* senescence), and partly mediate opposing functions (regarding survival [NF-κB] *vs.* apoptosis [p53]). Moreover, NF-κB promotes the secretion of pro-inflammatory cytokines, which are often found as part of the "senescence-associated secretory phenotype (SASP)". Lymphomas reflect a tumor entity in which both hyperactivating NF-κB mutations and inactivating *p53* mutations are frequent. However, the dynamic NF-κB/p53 interactions and the functional implications of distinct mutations or a deregulated NF-κB/p53 network are poorly understood. Utilizing a mouse lymphoma model and analyzing transcriptome and clinical data from lymphoma patients, we recently showed that therapy-induced senescence presents with and depends on active NF-κB signaling, and reapplied mouse model-derived genetic information to human data sets to define novel, clinically relevant patient subcohorts ("cross-species investigations"). We have now extended these analyses by modeling distinct lymphoma patient-derived NF-κB and p53 mutations in Eμ-myc transgenic mouse lymphomas. Preliminary data referring to tumor biology and treatment responses indicate common but also unique functional features of distinct NF-κB pathway as well as p53 lesions. Next, we aim to functionally dissect the NF-κB/p53 co-governed control of tumor growth, metabolism, cellular stress responses, and cross-

talk to the host immune system to identify novel vulnerabilities (as therapeutic "Achilles' Heels"), potential biomarkers of activity and drug action, and seek to mathematically model the relationship between NF-κB and p53.

Senescence-associated proteotoxicity accounts for a hypermetabolic phenotype that can be exploited for senescence-selective synthetic lethal therapies

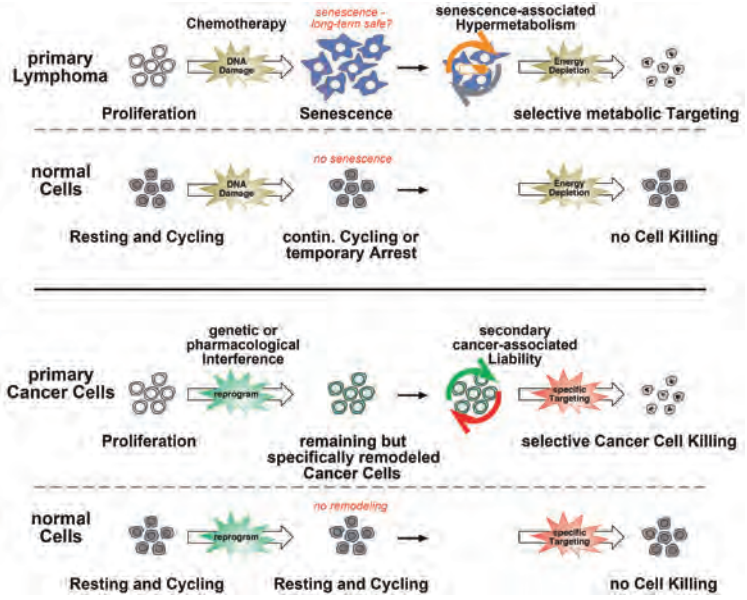
Jan Dörr, Yong Yu, Soyoung Lee, Jan Lisec and collaboration partners (Stefan Kempa et al.)

Functional non-invasive positron emission tomography (PET)-based imaging using either glucose or thymidine as tracers prompted the discovery of an unexpected feature of cellular senescence: a glucose-avid but thymidine-negative hypermetabolic phenotype. Extensive biochemical and genetic analyzes confirmed boosted glycolysis and oxidative phosphorylation in senescence, and led to the dissection of the underlying mechanism. Senescent cells present with a massive secretory phenotype (SASP, see above) that overwhelms the capacity of proper protein biosynthesis. As a consequence of endoplasmic reticulum stress and an unfolded protein response, senescent cells cope with proteotoxicity by energy-consuming autophagy. In turn, energy depletion or inhibition of autophagy results in selective cell death out of senescence, thereby utilizing senescence and these subsequent, conceptually novel treatment approaches in a "synthetically lethal-like" fashion to eliminate potentially harmful senescent cancer but no normal cells.

Chemotherapy promotes cancer stemness via senescence-related reprogramming

Maja Milanovic, Yong Yu, Henry Däbritz et al.

Many key signaling components of the senescence machinery also operate as critical regulators of stem cell functions. In cancer cells, altered stemness might have profound implications for tumor aggressiveness and clinical outcome. We investigated whether chemotherapy-induced senescence may change stem cell-related functionalities in malignant cells of murine and human origin.



Therapy-induced senescence as a cancer-exclusive condition that can be selectively targeted in a conceptually novel "next-generation synthetic lethal fashion". *A cancer-associated condition on one hand and a pharmacological targeting strategy on the other hand, where only their coincidence is cytotoxic.*

Selected Publications

Dörr, J.R., Y. Yu, M. Milanovic, G. Beuste, C. Zasada, J.H.M. Däbritz, J. Lisec, D. Lenze, A. Gerhardt, K. Schleicher, S. Kratzat, B. Purfürst, S. Walenta, W. Mueller-Klieser, M. Gräler, M. Hummel, U. Keller, A.K. Buck, B. Dörken, L. Willmitzer, M. Reimann, S. Kempa, S. Lee, and C.A. Schmitt. 2013. Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. **Nature**, 501:421-425.

Bywater, M.J., G. Poortinga, E. Sanij, N. Hein, A. Peck, C. Cullinane, M. Wall, L. Cluse, D. Drygin, K. Anderes, N. Huser, C. Proffitt, J. Bliesath, M. Haddach, M.K. Schwaebe, D.M. Ryckman, W.G. Rice, C. Schmitt, S.W. Lowe, R.W. Johnstone, R.B. Pearson, G.A. McArthur, and R.D. Hannan. 2012. Inhibition of RNA Polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. **Cancer Cell** 22:51-65.

Jing, H., J. Kase, J.R. Dörr, M. Milanovic, D. Lenze, M. Grau, G. Beuster, S. Ji, M. Reimann, P. Lenz, M. Hummel, B. Dörken, G. Lenz, C. Scheidereit, C.A. Schmitt, and S. Lee. 2011. Opposing roles of NF-kB in anti-cancer treatment outcome unveiled by cross-species investigations. **Genes Dev.** 25: 2137-2146.

Reimann, M., S. Lee, C. Loddenkemper, J.R. Dörr, V. Tabor, P. Aichele, H. Stein, B. Dörken, T. Jenuwein, and C.A. Schmitt. 2010. Tumor stroma-derived TGF-b limits Myc-driven lymphomagenesis via Suv39h1-dependent senescence. **Cancer Cell** 17: 262-272.

Braig, M., S. Lee, C. Loddenkemper, C. Rudolph, A.H.F.M. Peters, B. Schlegelberger, H. Stein, B. Dörken, T. Jenuwein and C.A. Schmitt. 2005. Oncogene-induced senescence as an initial barrier in lymphoma development. **Nature** 436: 660-665.

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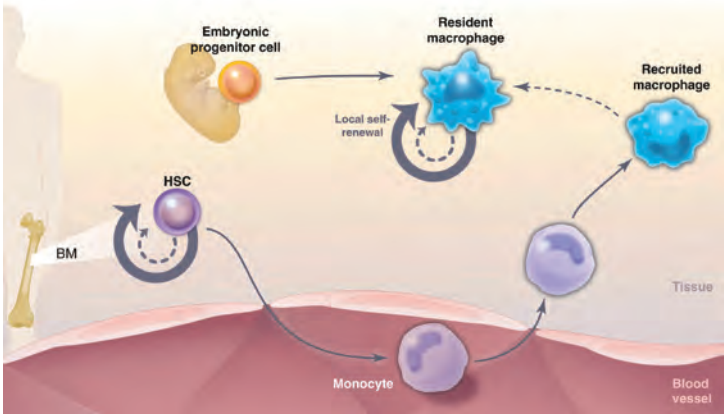


Photo: Jean-Marie Huron

Michael Sieweke

Stem Cell and Macrophage Biology

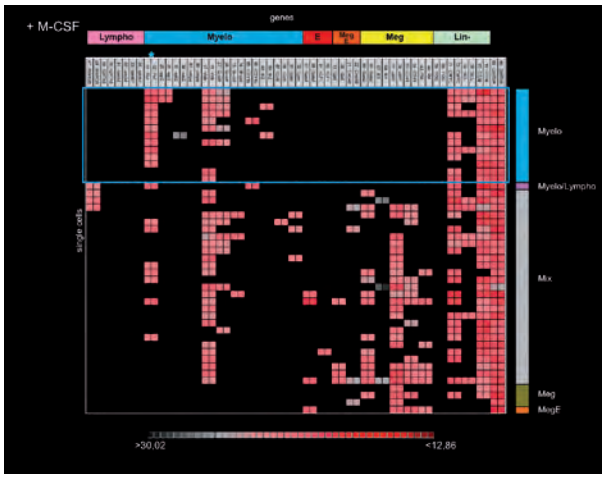
Our research is located at the interface of immunology and stem cell research with the overarching question of how cell identity and cell numbers are controlled. Often these processes appear intrinsically linked. By definition stem cells are immature and have not assumed specific effector functions but show an extended self-renewal capacity. As cells differentiate into specific functionally mature cells, self-renewal capacity is typically lost. Recent experiments have shown that cell identity is more malleable than previously thought and that the introduction of defined transcription factor cocktails can reprogram mature differentiated cells into pluripotent stem (iPS) cells or directly into other differentiated cells of other lineages. Essential for all projected stem cell applications, both for therapy and drug screening, is the generation of large numbers of cells with defined identity. Rather than manipulating cell identity we have taken the alternative and unique approach of identifying mechanisms that could induce self-renewal and thus increase cell numbers without change of cellular identity. Understanding the difference of such mechanisms to tumorigenic transformation would also be of critical importance for cancer research.



Origin and self-renewal of tissue macrophages
Katharine Sutliff © SCIENCE
Sieweke MH, Allen JE, Beyond Stem Cells : self-renewal of differentiated macrophages
Science, 22;342(6161) :1242974 (2013)

Learning from macrophages

To address these questions we investigate the molecular and cellular mechanisms of self-renewal and cell fate decision during the differentiation from hematopoietic stem cells to macrophages. These cells appear particularly suited to identify self-renewal mechanisms independent of lineage conversion since tissue macrophages cannot only originate from hematopoietic stem cells via blood monocyte intermediates but can also be derived from embryonic progenitors and be maintained long term in tissues without monocyte input. This may involve stem-cell like self renewal mechanisms (reviewed in Sieweke and Allen, Science 2013; figure 1). Indeed we could show that inactivation of two macrophage specific transcription factors enabled long term self-renewal of macrophages without loss of cell identity or tumorigenic transformation (Aziz et al., Science 2009). Furthermore macrophages have recently entered the limelight of immunology and beyond (Geissman et al., Science 2010), because they are involved in many physiological and pathological processes of major interest beyond their immune functions, such as regeneration, cancer and metabolic diseases. Macrophages are present in essentially every tissue of the body, where they can play trophic roles in tissue homeostasis, metabolism and repair. Macrophages could thus be considered systemic tissue guardians, whose function and manipulation could have major impact on health and disease of parenchymal cells.



***Single cell gene expression analysis of HSC after 16h culture with M-CSF**. The genes analyzed are classified and color coded on top. Cells are clustered according to lineage identity identicated on the right.*

In this framework we have developed research projects investigating the lineage commitment of hematopoietic stem cells to the macrophage lineage, self-renewal and cellular identity of mature macrophages and the role of macrophage activation states in inflammation and tissue regeneration.

Lineage commitment of hematopoietic stem cells

We have investigated the very first steps of monocyte differentiation that are initiated by myeloid lineage commitment in hematopoietic stem cells (HSC). Previously it had been widely assumed that cell fate choice in stem cells is largely determined by stochastic mechanisms. By the use of multiple methods of single cell analysis (fig.2) in vitro and in vivo we could demonstrate that the cytokine M-CSF can instruct highly purified HSC to adopt a myeloid fate (Mossadegh,Sarrazin et al., Nature 2013). We also showed that this is mediated by an integrated circuit of the transcription factor MafB with M-CSF, where MafB acts as a threshold setter for M-CSF driven asymmetric commitment divisions (Sarrazin et al., Cell 2009). These findings resolved a long-standing debate about the capacity of cytokines to instruct cell fate and about the coordination of cell intrinsic and extrinsic regulators in lineage choice. These studies have important implications for stem cell differentiation in general beyond the hematopoietic system.

Self-renewal of differentiated cells

Terminal differentiation is typically tightly linked to cell cycle withdrawal. However, we discovered that MafB/cMaf deficiency enables self-renewal of differentiated functional macrophages by a cMyc/KLF4 dependent mechanism. It thus appears possible to amplify functional differentiated cells without malignant transformation or stem cell intermediates (Aziz et al, Science 2009). We currently investigate the mechanisms of self-renewal in differentiated cells and explore the therapeutic potential of amplified macrophages and their utility as a research tool.

Macrophages in tissue regeneration

Inflammation and tissue regeneration are often linked processes but require precise timing and coordination of different cell types. In this context we investigate the cell fate decisions that give rise to different macrophage subtypes. We want to identify the transcription factors controlling these choices and aim to understand how their manipulation affects tissue repair.

Patents / Patent applications

Method for expanding monocytes
United States patent US 8,574,903 B2, filed 10.01.2008, delivered 05.11.2013

Method for generating, maintaining and expanding monocytes, and/or macrophages and/or dendritic cells in long term culture
European patent EP 07300717.1.-2405; filed 10.01.2007
United States patent PCT/EP2008/050221;filed 10.01.2008, delivered 08.04.2014

A method for inducing extended self-renewal of functionally differentiated somatic cells
European patent EP 10734946.6-2401: filed 08.07.2009
United States patent PCT/EP2010/059843:filed 08.07.2010

Methods and compositions for use in preventing or treating myeloid cytopenia and related complications
European patent EP 13305464.3-1456: filed 09.04.2013

MafB mutants and uses thereof
European patent EP13306661.3: filed 03.12.2013

Selected Publications

Sieweke MH, Allen JE, **Beyond stem cells: self-renewal of differentiated macrophages, Science**, 2013, 22;342(6161): 1242974

Mossadegh-Keller N, Sarrazin S, Kandalla PK, Espinosa L, Stanley RE, Nutt SL, Moore J, Sieweke MH, **M-CSF instructs myeloid lineage fate in single haematopoietic stem cells, Nature,** 2013, 9;497(7448):239-43

Geissmann F, Manz M, Jung S, Sieweke M, Merad M, Ley K, **Development of monocytes, macrophages and dendritic cells, Science**, 2010, 327 (5966):656-61

Aziz A, Soucie E, Sarrazin S, Sieweke MH, **MafB/c-Maf deficiency enables self-renewal of differentiated functional macrophages, Science**, 2009, 326 (5954):867-71

Sarrazin S, Mossadegh-Keller N, Fukao T, Aziz A, Mourcin F, Kelly L, Vanhille L, Kastner P, Chan S, Duprez E, Otto C. Sieweke MH, **MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells, Cell**, 2009, 138(2):300-13

July 2012: Start of the franco-german research group with the Centre d'Immunology de Marseille-Luminy, Marseille, France, as part of a new Helmholtz-INSERM Franco-German research cooperation initiative

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Photo: David Ausserhofer/MDC

Thomas Sommer

Video:
How are unnecessary proteins degraded in cells?

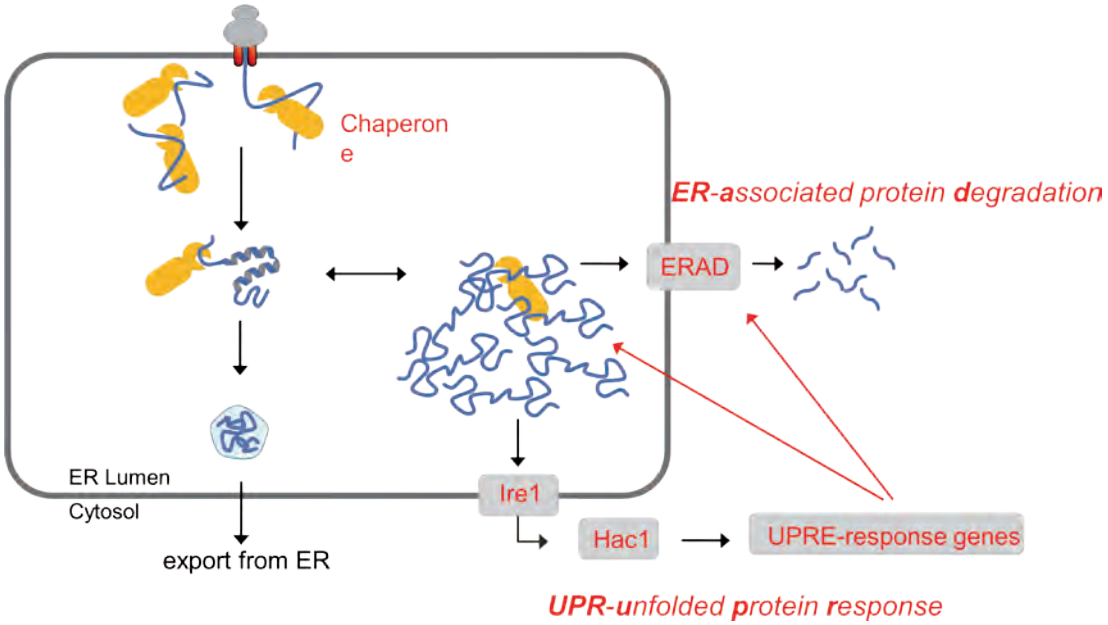
Intracellular Proteolysis

The Mechanisms of Protein Quality Control

In all cells, both newly synthesized and pre-existing proteins are constantly endangered by misfolding and aggregation. The accumulation of such damaged proteins perturbs cellular homeostasis and provokes aging, pathological states, and even cell death. To avert these dangers, cells have developed protein quality control (PQC) strategies that counteract protein damage in a compartment-specific manner. PQC systems channel misfolded proteins either into re-folding pathways or initiate their proteolysis by the Ubiquitin Proteasome System (UPS). The work of this group focuses on how specific branches of the UPS selectively dispose aberrant proteins without affecting correctly folded polypeptides.

One of the best investigated PQC pathways exist in the endoplasmic reticulum (ER). The ER is a cellular organelle through which a large number of proteins travel on their way to their final destination in membranes, exocytic and endocytic compartments, or the cell exterior. Since proteins enter the ER in an unfolded conformation, this organelle is a major folding compartment of eukaryotic cells. To fulfill this task, it hosts an array of molecular chaperones, which assist in protein folding and maturation. Still, protein biogenesis is an error-prone process. A considerable fraction of all newly synthesized polypeptides fail to attain their native conformation due to mutations, transcriptional and translational errors, folding defects, or imbalanced subunit synthesis. Mature proteins can be damaged by environmental stress conditions, such as high-energy radiation, chemical insults, or metabolic by-products. Specific components of the UPS eliminate such misfolded proteins in a process termed ER associated protein degradation (ERAD). The ERAD pathway cooperates with the Unfolded Protein Response (UPR) in maintaining homeostasis in the ER. The UPR measures the degree of misfolding in the ER and regulates folding and proteolytic capacities according to the load with aberrant proteins (Figure 1).

ERAD appears to be conserved from yeast to mammals. Thus, many basic principles and components have been discovered in the model organism Saccharomyces cerevisiae. One of the highly conserved components of the ER is the HRD ubiquitin ligase. This membrane-bound complex recognizes signals contained in misfolded proteins of the ER-lumen and ER-membrane. Proteins



Protein homeostasis in the endoplasmic reticulum. *Proteins translocate into the ER via the narrow protein translocation channel. Newly synthesized and unfolded proteins associate with molecular chaperones to fold. Only correctly folded proteins leave the ER for their final destination. Misfolded proteins are retained in the ER and either channeled again into folding pathways or subjected to protein dislocation initiated by UPS components (ERAD pathway). The UPR component Ire1 measures the content of misfolding in the ER, signals it to the nucleus, and regulates expression of a multitude of genes. This transcriptional program includes up-regulation of genes coding for molecular chaperones and UPS components of the ERAD branch.*

committed for degradation are exported from the ER in a process termed protein dislocation. Subsequently, dislocated substrate molecules are ubiquitylated and degraded by cytoplasmic 26S proteasomes (Figure 2).

In the last decade, the group has identified and characterized components of ligase complexes using genetics, molecular biology, and protein purification strategies. However, this laboratory has now established powerful in vitro approaches and quantitative methods using purified components to understand the biochemical principles of the ERAD pathway.

Hrd3 and Yos9 possess 'Holdase' activity and function as substrate receptors of the HRD ligase.

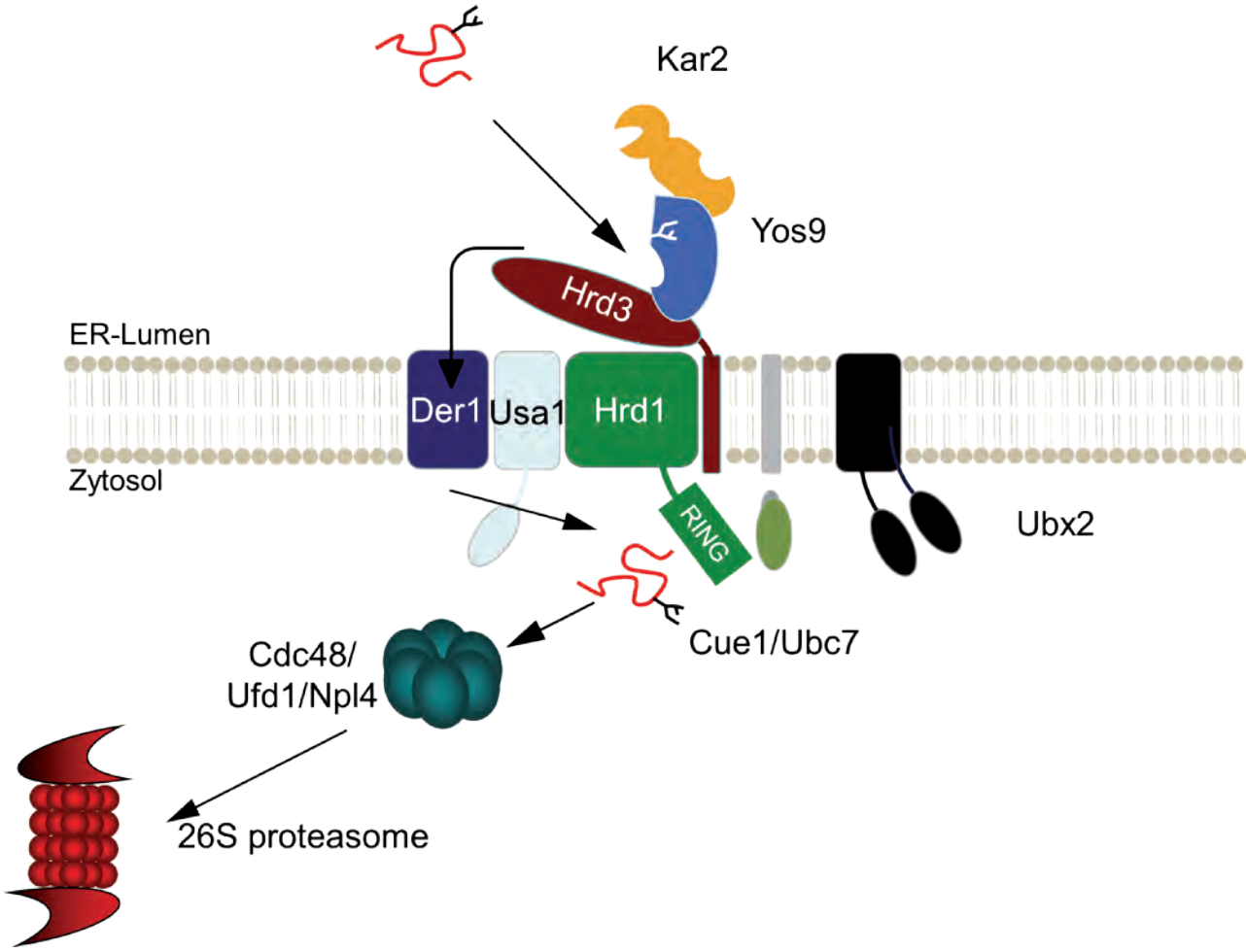
Franziska Zimmermann, Sathish Lakshimpathy and Maren Berger

In order to understand how substrates bind to the ER-luminal module of the HRD ligase (Hrd3, Yos9) we started to work with purified components. Since the Hrd3 function does only rely on its ER-luminal domain we expressed the luminal domain in insect cells. We also expressed Yos9 in E. coli or insect cells. Expressed and purified Hrd3 and Yos9 bind directly denatured firefly luciferase but not the folded enzyme. Both Hrd3 and Yos9 deploy holdase activity as they prevent the aggregation of a denatured protein. Our in vitro data imply that the HRD ligase directly recognizes misfolding of proteins.

A Ubiquitin-Binding Domain regulates ubiquitin chain synthesis.

Katrin Bagola and Maximilian von Delbrück

Ubiquitin-binding domains (UBD) recognize ubiquitin (ub) modifications. Some of them specifically bind mono-ub while others recognize specifically linked poly-ubiquitin chains. They are crucial for the UPS since they could 'read' the ubiquitin code. Thus far, no significant ubiquitin binding has been observed for the UBD of yeast



The Hrd ubiquitin ligase as an example of a quality control element. *The ER-luminal modul of the ligase consists of Hrd3 and Yos9 and associates with the Hsp70 type chaperone Kar2. Der1, Usa1, and the transmembrane (TM) domains of Hrd1 collectively build the membrane module of the ligase. Some of these TM regions are most likely involved in building a pore in the ER-membrane through which misfolded proteins are dislocated. The cytosolic module consisting of Hrd1 and Cue1/Ubc7 modifies dislocated proteins with K48 linked poly-ubiquitin chains. These chains and Ubx2 recruit the AAA-ATPase Cdc48 and its co-factors Ufd1 and Npl4 to the ligase complex. Cdc48 most likely provides energy for the dislocation event and hands-over the ubiquitylated proteins to the 26S proteasome for degradation.*

Cue1, the CUE domain. Cue1 integrates the E2 enzyme Ubc7 into membrane-bound ubiquitin ligases at the Endoplasmic Reticulum (ER) and it furthermore activates it. Therefore, Cue1 is crucial for ER-associated protein degradation (ERAD). We were able to provide evidence that the CUE domain of Cue1 binds oligo-ubiquitin chains, which is pivotal for the efficient synthesis of K48-linked poly-ubiquitin chains in vitro. In addition we have identified mutations that abolish ubiquitin binding by Cue1 and affect turnover of ERAD substrates in vivo. Our data point strongly to a molecular mechanism in which the CUE domain positions the tip of a growing ubiquitin chain in close proximity to the active site center of the E2 enzyme to promote efficient chain elongation (Bagola et al, Mol Cell, 2013).

The function of other Ubiquitin-conjugating enzymes.

Katrin Bagola, Annika Weber and Lukas Pluska

In addition to Ubc7 and Cue1 other ubiquitin-conjugating enzymes function in ERAD as well. For example Ubc1 works together with Hrd1 in a parallel pathway to Cue1/

Ubc7. Preliminary results indicate specific and novel mechanisms how this E2 enzyme promotes ubiquitin chain formation.

Ubc6 cooperates with the Doa10 ubiquitin ligase and also in this case we are investigating the molecular mechanisms how this enzyme contributes to ubiquitin chain formation on substrates of this ligase complex at the ER.

A channel for ERAD in the ER-membrane.

Martin Mehnert and Ernst Jarosch

Dislocation of selected misfolded proteins through the lipid bilayer is assumed to occur via a protein-conducting channel of unknown nature. We were able to show that the integral membrane protein Der1 oligomerises which relies on its interaction with the scaffolding protein Usa1. Mutations in the transmembrane domains of Der1 block the passage of soluble proteins across the ER-membrane. As determined by site-specific in vivo photocrosslinking, the ER-luminal exposed parts of Der1 are in spatial proximity to the substrate receptor Hrd3 whereas the membrane-embedded domains reside adjacent to the ubiquitin ligase Hrd1. Intriguingly, both regions also form crosslinks to client proteins. In summary our data imply that Der1 initiates the export of aberrant polypeptides from the ER-lumen by threading such molecules into the ER-membrane and routing them to Hrd1 for ubiquitylation (Mehnert et al., Nature Cell Biol., 2014).

Htm1p is a mannosidase that generates N-linked Man7GlcNAc2 glycans to accelerate the degradation of misfolded glycoproteins.

Anett Köhler and Christian Hirsch

N-linked glycans are essential for the breakdown of glycoproteins. The covalently attached oligosaccharide structure is used as a signal indicating the folding status of the protein. Newly synthesized proteins receive a Glc3Man9GlcNAc2 modification. This glycan structure is trimmed by glucosidases and mannosidases until a specific signal is generated (Man7GlcNAc2), which is recognized by the HRD ubiquitin ligase. Htm1p is a mannosidase in this trimming pathway that interacts with the oxidoreductase Pdi1. Using an in vitro approach as well as molecular biology approaches this Delbrück group investigates how the heterodimer of Htm1 and Pdi1 recognizes quality control substrates and trims their oligosaccharides to make them visible for downstream acting factors.

Selected Publications

Horn, S.C., Hanna, J., Hirsch, C., Volkwein, C., Schütz, A., Heinemann, U., Sommer, T., and Jarosch, E. (2009) Usa1 functions as a scaffold of the HRD ubiquitin ligase. **Mol Cell** 36, 782-793.

Buchberger, A, Bukau, B, and Sommer, T. (2010) Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. **Mol Cell** 40, 238-252.

Shabek, N., Herman-Bachinsky, Y., Buchsbaum, S., Lewinson, O., Haj-Yahya, M., Hejjaoui, M., Lashuel, H. A., Sommer, T., Brik, A., and Ciechanover, A. (2012) The size of the proteasomal substrate determines whether its degradation will be mediated by mono- or polyubiquitylation. **Mol Cell** 48, 87-97.

Bagola, K., von Delbrück, M., Dittmar, G., Scheffner, M., Ziv, I., Glickman, M. H., Ciechanover, A., and Sommer, T. (2013) Ubiquitin Binding by a CUE Domain Regulates Ubiquitin Chain Formation by ERAD E3 Ligases. **Mol Cell** 50, 528-539.

Mehnert, M., Sommer, T., and Jarosch, E. (2014) Der1 promotes movement of misfolded proteins through the endoplasmic reticulum membrane. **Nature Cell Biol** 16, 77-86

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Photo: David Ausserhofer/MDC

Wolfgang Uckert

Molecular Cell Biology and Gene Therapy

Over the past decade, the genetic introduction of T cell receptor (TCR) genes into T cells has been developed as a strategy to provide defined antigen-specific T cell immunity. TCR gene transfer aims to target tumor or virus-infected cells by genetic modification of T cells. The potential value of this strategy - designated as TCR gene therapy – was established in mouse models and phase I clinical trials. However, most studies were of limited efficacy and accompanied by side effects and revealed i), the importance to select the right candidate as target antigen and ii), the need to improve the methodology for TCR gene therapy. We address questions related to: generation of TCR-modified T cells with new antigen specificity and high functional avidity, safety aspects of TCR-modified T cells with respect to on- and off-target effects, adoptive transfer of TCR-modified T cells in preclinical models and the use of TCR-modified Treg in the treatment of autoimmune diseases.

Multi-functional vectors for TCR gene therapy

For clinical application TCR gene transfer into T cells needs to be i) efficient and specific, ii) safe and iii) cost-effective. To this end, our group develops highly sophisticated gene transfer vectors based on retroviral or transposon systems.

Inan Edes in collaboration with C. Buchholz (PEI, Langen)
In collaboration with the group of C. Buchholz we have constructed lentiviral vectors, which allow targeted gene transfer specifically into human and murine $CD8^+$ or $CD4^+$ T cell subsets. These vectors were pseudotyped with measles virus-derived envelopes whose specificity was redirected by CD4- or CD8-specific single-chain antibodies and which were blinded for their native receptors. Such subset-specific vectors allow the targeted delivery of MHC class I- and class II-restricted TCRs into $CD8^+$ or $CD4^+$ T cells without the need for cell sorting. Further, they can be employed for T cell subset-specific expression of immune-modulating transgenes, which could enhance anti-tumor efficacy. Finally, *in vivo* application of targeting vector particles would abrogate the need for *ex vivo* T cell transduction and expansion.

Mario Bunse
In TCR-modified T cells the transduced TCRα/β heterodimer can form potentially self-reactive mixed TCR dimers, composed of endogenous and transferred TCR chains, which have been demonstrated to cause lethal autoimmunity in a mouse model of TCR gene therapy. To prevent mixed TCR dimer formation, we developed an RNAi-TCR replacement vector that simultaneously silences the endogenous TCR and expresses an RNAi-resistant, therapeutic TCR. Importantly, lethal TCR gene transfer-induced autoimmunity caused by mixed TCR dimer formation was almost completely prevented when the cells were transduced using the RNAi-TCR replacement vector. Presently, we are adapting this technology for the silencing of human endogenous TCR. Furthermore, we constructed a transposon vector analogous to the viral RNAi-TCR replacement vector.

Julian Clauß in collaboration with Z. Izsvak (MDC, Berlin)
Currently, most clinical protocols use viral vectors for gene transfer into T cells. However, GMP manufacturing of DNA plasmids is cheaper and faster compared to the production of retroviral supernatants and plasmid vectors have no strict size limitation of the expression cassette. The DNA-based Sleeping Beauty transposon system (SBTS) enables stable integration of the transgene cassette into the genome of primary human T cells by

nucleofection. Using the optimized hyperactive transposase SB100Xo we gene-modified primary human T cells yielding high and long-term transgene expression with integration rates of approx. 45% (GFP) or 30% (TCR). Cell viability after nucleofection varied between 20% and 60%. Provision of the transposase as *in vitro*-transcribed RNA (ivtRNA) instead of plasmid DNA resulted in increased gene transfer efficacy and improved T cell viability. SBTS-mediated TCR gene transfer generated T cells with tumor cell-directed reactivity as measured by IFNγ release after co-cultivation of TCR-modified T cells and antigen-presenting cells. This technology will be further developed into a robust non-viral vector platform to generate tumor-specific T cells for personalized cancer immunotherapy.

Pre-clinical *in vivo* models to test the efficacy of TCR gene therapy

Matthias Leisegang and Peter Meyerhuber in collaboration with Thomas Blankenstein (MDC, Berlin), Helga Bernhard (Darmstadt), Dolores Schendel (Helmholtz Zentrum, Munich)

We developed mouse models to evaluate the therapeutic efficiency and safety of TCR gene therapy for established cancer. We generated immune-deficient, human MHC-transgenic mice (HHDxRag$^{-/-}$) and a syngeneic tumor cell line that can be modified to express human tumor antigens of interest. T cells engineered with human tyrosinase-specific TCR of different affinities were adoptively transferred into mice bearing tyrosinase-positive tumors. While transfer of T cells engineered with a high-affinity tyrosinase-specific TCR resulted in complete tumor rejection, low TCR affinity selected for escape variants and relapse. In a second mouse model (NOD-SCIDxHHD) T cells transduced with a panel of TCR recognizing human epidermal growth factor receptor 2 (HER2) with different affinities are tested for i) their efficacy in rejecting large established human HER2-positive tumors and ii) their on-target toxicity on HER2-expressing normal tissue. This latter is possible as humans and mice share the same HER2-dervied epitope that is recognized by these TCRs. These *in vivo* models provide a versatile, pre-clinical test system for TCR gene therapy of cancer and allows the prediction whether or not TCR-engineered T cells eradicate tumors or select escape variants and whether the targeted antigen is safe.

TCR gene transfer into regulatory T cells for the suppression of autoimmune disease

Elisa Kieback in collaboration with Simon Fillatreau (DRFZ, Berlin)

The function of regulatory T cells (Treg) is to limit the extent of immune reactions and to suppress self-reactivity. Activation of endogenous Treg or adoptive transfer of *in vitro* expanded Treg has been applied to treat various autoimmune disorders. Several studies have demonstrated that therapeutic efficacy depends on their antigen-specificity. A novel method to generate high numbers of antigen-specific Treg is the transfer of TCR genes into the cells.

We have transduced the genes encoding TCR directed against the CNS antigen myelin oligodendrocyte glycoprotein (MOG) into Treg. TCR-modified Treg could suppress proliferation of effector T cells *in vitro* and adoptive transfer of very low numbers of TCR-modified Treg – but not polyclonal Treg – could inhibit development of MOG peptide-induced experimental autoimmune encephalitis (EAE) in mice. The therapeutic effect was dependent on high affinity of the TCR and expression of CTLA-4 by the Treg as Tregs transduced with low-affinity TCRs or a miRNA targeting CTLA-4 were not able to suppress EAE upon transfer.

Selected Publications

Reuss S, Sebestyen Z, Heinz N, Loew R, Baum C, Debets R, Uckert W (2014). TCR-engineered T cells: A model of inducible TCR expression to dissect the interrelationship between two TCRs. Eur J Immunol 44: 265-274.

Stärck L, Popp K, Pircher H, Uckert W (2014). Immunotherapy with TCR-redirected T cells: comparison of TCR-transduced and TCR-engineered hematopoietic stem cell-derived T cells. J Immunol 192: 206-213.

Sommermeyer D, Conrad H, Kronig H, Gelfort H, Bernhard H, Uckert W (2013). NY-ESO-1 antigen-reactive T cell receptors exhibit diverse therapeutic capability. Int J Cancer 132: 1360-1367.

Zhou Q, Schneider IC, Edes I, Honegger A, Bach P, Schönfeld K, Schambach A, Wels WS, Kneissl S, Uckert W, Buchholz CJ (2012). T-cell receptor gene transfer exclusively to human CD8(+) cells enhances tumor cell killing. Blood 120: 4334-4342.

Bendle GM, Linnemann C, Hooijkaas AI, de Witte MA, Jorritsma A, Bies L, Kaiser ADM, Pouw N, Debets R, Kieback E, Uckert W, Song J-Y, Haanen JBAG, Schumacher TNM. (2010).Lethal Graft-versus-host disease in mouse models of T cell receptor gene therapy. Nature Med 16: 565-570.

Structure of the Group

Group Leader
Prof. Dr. Wolfgang Uckert

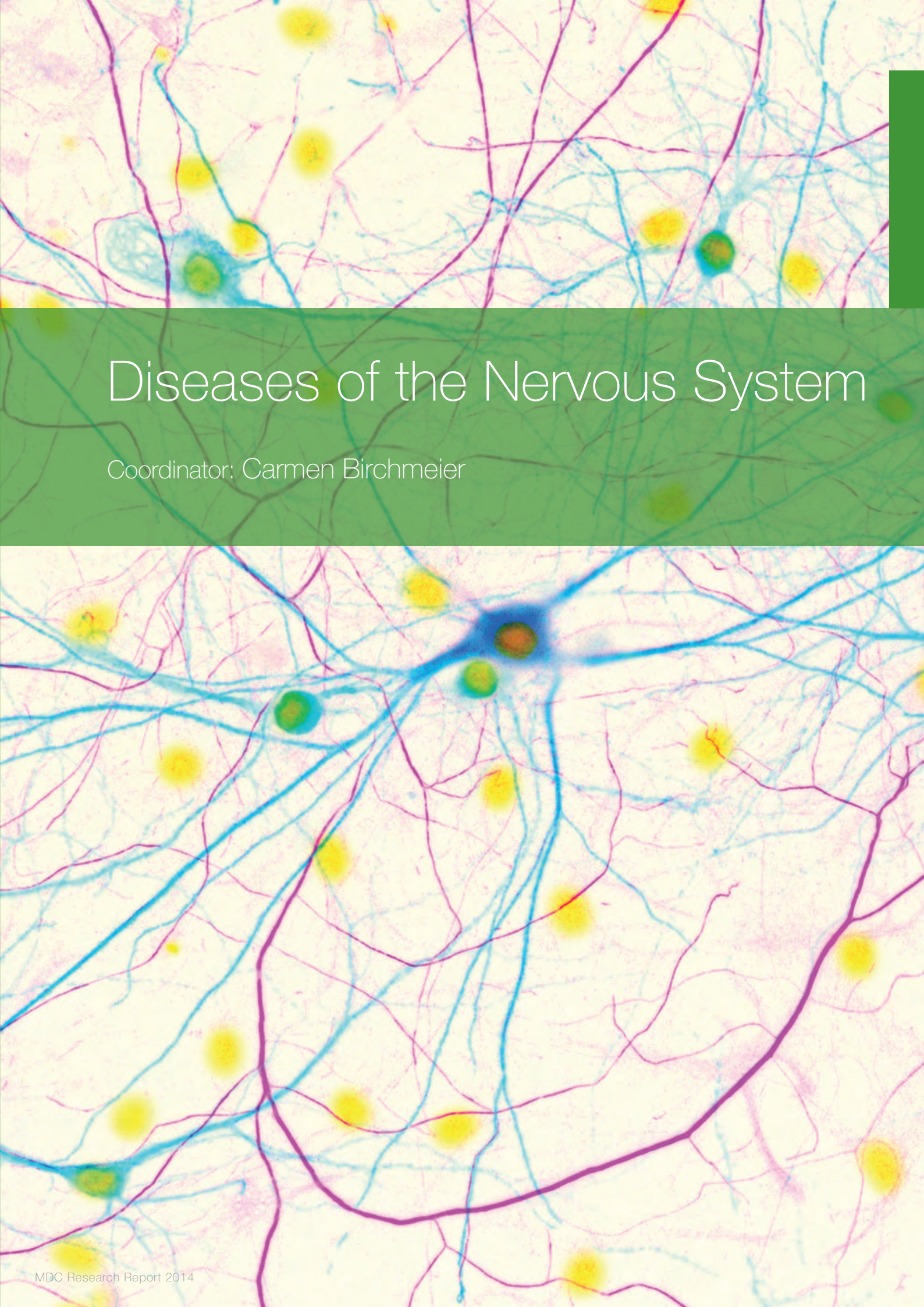
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Dr. Matthias Leisegang
Dr. Lilian Staerck

PhD
Mario Bunse
Julian Clauß
Inan Edes
Felix Lorenz
Peter Meyerhuber

Graduate and Undergraduate
Maria Lietz

Technical Assistants
Carolin Genehr
Janina Hauchwitz
Kordelia Hummel
Mareen Kamarys
Michaela Naschke
Melanie Schwochow

Other
Erika von Buehler (SFB TR36)



Diseases of the Nervous System

Coordinator: Carmen Birchmeier

Diseases of the Nervous System

Carmen Birchmeier

The nervous system is fundamental to the body's ability to maintain itself, to sense the environment, to move and react to stimuli, and to generate and control behavior. Hence, disorders of the nervous system that manifest themselves as neurological and psychiatric disease often severely impair afflicted individuals and impose a heavy burden on patients, families, caregivers and society alike. Research in the Neuroscience Program of the MDC focuses on determining the molecular basis of normal nervous system function, and discovering changes responsible for inherited or acquired defects are important priorities in the fight against nervous system disease.

Neuroscientists of the MDC are important participants in many Berlin-based cooperative networks (e.g. several Collaborative Research Centers, Transregios and Research Units funded by the DFG). Seven of the eleven principle investigators of the program are associated with NeuroCure, the Berlin-based excellence cluster in neuroscience. NeuroCure was evaluated and will receive funding for a second period (2012-2017). In addition, many of the groups received grants from the European Union, in particular **Gary Lewin** and **Thomas Jentsch** were awarded ERC advanced grants.

The success of the Neuroscience Program is also reflected in the fact that young researchers received offers for independent positions from outside and left the MDC. **Alistair Garratt** became Associate professor (Charité) in 2012, **Stefan Lechner** became independent group leader (University of Heidelberg) in 2013, and **Jan Siemens** was appointed extraordinary professor (University of Heidelberg). On the other side, two new principle investigators joined the Program, **Kate Poole** (2013) and **Annette Hammes** (2014).

Scientists from the Neuroscience Program participated in research and graduate schools, among them the **Helmholtz International Research School MolNeuro,** the **Charité International Medical Neurosciences** program, and **MyoGrad**. The curriculum of these Graduate Schools encompasses lecture series, journal clubs, courses, and they fund students to attend international conferences.

Noteworthy are also other honors received, for instance **James Poulet** obtained the Paul Ehrlich und Ludwig Darmstaedter Award for Junior Scientists and **Helmut Kettenmann** was elected President of the German Neuroscience Society, and **Thomas Jentsch** became member of the Committee of the Louis-Jeantet Foundation. **Scientific highlights from the Department**

Patients with high grade gliomas have a very poor prognosis because these tumors are rarely treatable. **Rainer Glass** and **Helmut Kettenmann** found that endogenous neural precursor cells exert anti-tumour activity by activating the vanilloid receptor TRPV1 on glioma cells, thus identifying TRPV1 agonists as potential glioma therapeutics. (Stock et al., Nat Med. 2012).
In vertebrates, hearing and touch both rely on the detection of mechanical stimuli. Using a classical twin study design, the group of **Gary Lewin** found a significant correlation between genes governing touch and hearing acuity in healthy humans. They also identified one gene that contributes to both sensory systems, USH2A (Frenzel et al., PLoS Biol. 2012).

The sense of touch relies on detection of mechanical stimuli by specialized mechanosensory neurons. The groups of **Carmen Birchmeier** and **Gary Lewin** showed that the transcription factor c-Maf is crucial for mechanosensation in mice and humans. Pacinian corpuscles are specialized to detect high-frequency vibration and are atrophied in c-Maf mutant mice. In line with this, sensitivity to high-frequency vibration

is reduced in humans carrying a dominant mutation in c-MAF. (Wende et al., Science 2012).

Mutations inactivating the potassium channel KCNQ4 lead to deafness in humans and mice. The groups of **Thomas Jentsch** and **Gary Lewin** have detected KCNQ4 in the peripheral nerve endings of cutaneous mechanoreceptors from mice and humans. Electrophysiological analyses of mice demonstrated elevated mechanosensitivity in KCNQ4 mutant mice, similar to the increased vibrotactile acuity observed in patients that lack a functional KCNQ4 gene (Heidenreich et al., Nat. Neuroscience 2011).

Proteins with long polyglutamine sequences have an enhanced propensity to self-assemble into disease-causing insoluble protein aggregates. The inherited neurodegenerative disease Spinocerebellar Ataxia Type 1 is caused by mutations in ataxin-1. The group of **Erich Wanker** has identified 21 human proteins that influence ataxin-1 misfolding; some enhance and others suppress misfolding and aggregation (Petrakis et al., PLoS Genetics 2012).

High blood pressure is the leading risk factor for death worldwide. The group of **Björn Schröder,** in collaboration with Christian A. Hübner (Jena), identified a new ion channel, TMEM16A, as an important calcium-activated chloride channel that controls peripheral vascular resistance. Their data data suggest that TMEM16A is a promising target for hypertension treatment (Heinze et al., J. Clin. Invest., 2014).

Axonal branching is a prerequisite for formation of complex neuronal circuits and for parallel information processing. **Fritz Rathjen** and **Hannes Schmidt** foundthat Npr2 activity is needed for bifurcation of cranial sensory axons, with axons only turning in either an ascending or descending direction instead of branching when they enter the central nervous system (Ter-Avetisyan et al., J. Neuroscience 2014).

The inhibitory neurotransmitter whose effects are mediated by glycine receptors (GlyR). The group of **Jochen Meier** discovered a gain-of-function variant of GlyR in the brain of epileptic patients. They now expressed this variant GlyR in mice in an neuron type-specific pattern: expression in glutamatergic neurons resulted in recurrent epileptiform discharge, whereas expression in inhibitory interneurons resulted in increased anxiety. The work identifies the pathophysiological mechanisms in epilepsy (Winkelmann et al., J. Clin. Invest., 2014).

Cortical circuits spontaneously generate coordinated activity even in the absence of external inputs. **James Poulet** and his collaborators in the group of Carl Peterson investigated the impact of the thalamus on cortical activity. They demonstrate that the desynchronized cortical state is driven by an increase in thalamic action potential firing, which is mimicked by optogenetic stimulation of the thalamus (Poulet et al., Nat Neurosci. 2012).

Sonic hedgehog (SHH) is a regulator of forebrain development that acts through the patched 1 receptor. The group of **Annette Hammes** and Thomas Willnow identified LRP2, a member of the LDL receptor gene family, as a component of SHH signaling. The data reveal the molecular mechanism underlying congenital forebrain anomalies (Christ et al., Dev. Cell 2012).

In sensory neurons, mechanotransduction is sensitive, fast and requires mechanosensitive ion channels. **Kate Poole** developed a new method to directly monitor mechanotransduction at defined regions of the cell-substrate interface (Poole et al, Nat. Comm. 2014).


Photo: Thomas Müller

Carmen Birchmeier

Developmental Biology / Signal Transduction

Our lab primarily focused on the functional analysis of genes important in embryonic development and adult stem cells, concentrating on muscle, the nervous system and endocrine cells. For our work, we use mouse genetics and cell culture analyses of stem cells, and define molecular mechanisms using genome and proteome technologies.

Muscle stem cells and their niche

Dominique Bröhl, Elena Vasyutina, Maciej T. Czajkowski, Joscha Griger, Claudia Rassek, Hagen Wende, in collaboration with Hans-Peter Rahn (Preparative Flow Cytometry Facility, MDC) and Bettina Purfürst (Electron Microscopy Core Facility, MDC).

Skeletal muscle growth and regeneration rely on myogenic progenitor cells. Satellite cells are the progenitor/stem cells of the postnatal muscle and they reside between basal lamina and plasma membrane of myofibers. This anatomical position is called the stem cell niche. Previous data show that generation and maintenance of satellite cells depends on Notch signals. For instance, elimination of Rbpj, the transcriptional mediator of Notch signals, results in premature differentiation of myogenic progenitors and the formation of tiny muscle groups that lack satellite cells (Vasyutina et al., 2007). We recently found that this drastic effect of the loss of Notch signals is rescued by the additional mutation of the muscle differentiation factor MyoD. Thus, a major function of Notch during fetal myogenesis is the repression of MyoD function.

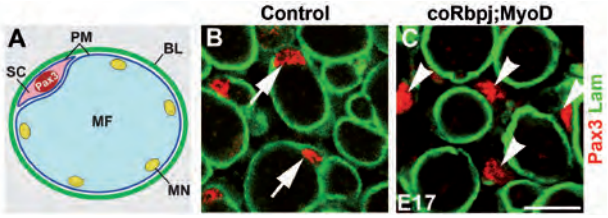


Fig. 1 Muscle stem cells require Notch signaling to settle in their niche.
(A) Schematic diagram showing the anatomical localization of the stem cells of the muscle that are also called satellite cells (SC, red) wedged between the basal lamina (BL) and the myofiber (MF) plasma membrane (PM). Satellite cells nuclei express Pax3/Pax7 and are shown in red, nuclei of the myofiber (myonuclei, MN) are shown in yellow. (B,C) Analysis of emerging satellite cells at E17.5 using antibodies against Pax3 (red) and laminin (green). Shown are sections of back muscle from control and Rbpj; MyoD mutant mice. Arrows point towards satellite cells located below the basal lamina, and arrowheads point towards the Pax3+ cells located in the interstitial space of Rbpj/MyoD double mutants.

However, rescued myogenic progenitors in the Rbpj/MyoD double mutants do not assume a satellite cell position (Fig. 1). Instead, they locate to the interstitial space of the muscle and contribute poorly to myofiber growth. Our work shows that Notch signaling stimulates emerging satellite cells to adhere to myofibers and to contribute to the production of the basal lamina that eventually surrounds both, the satellite cell and the myofiber (Bröhl et al., 2012).

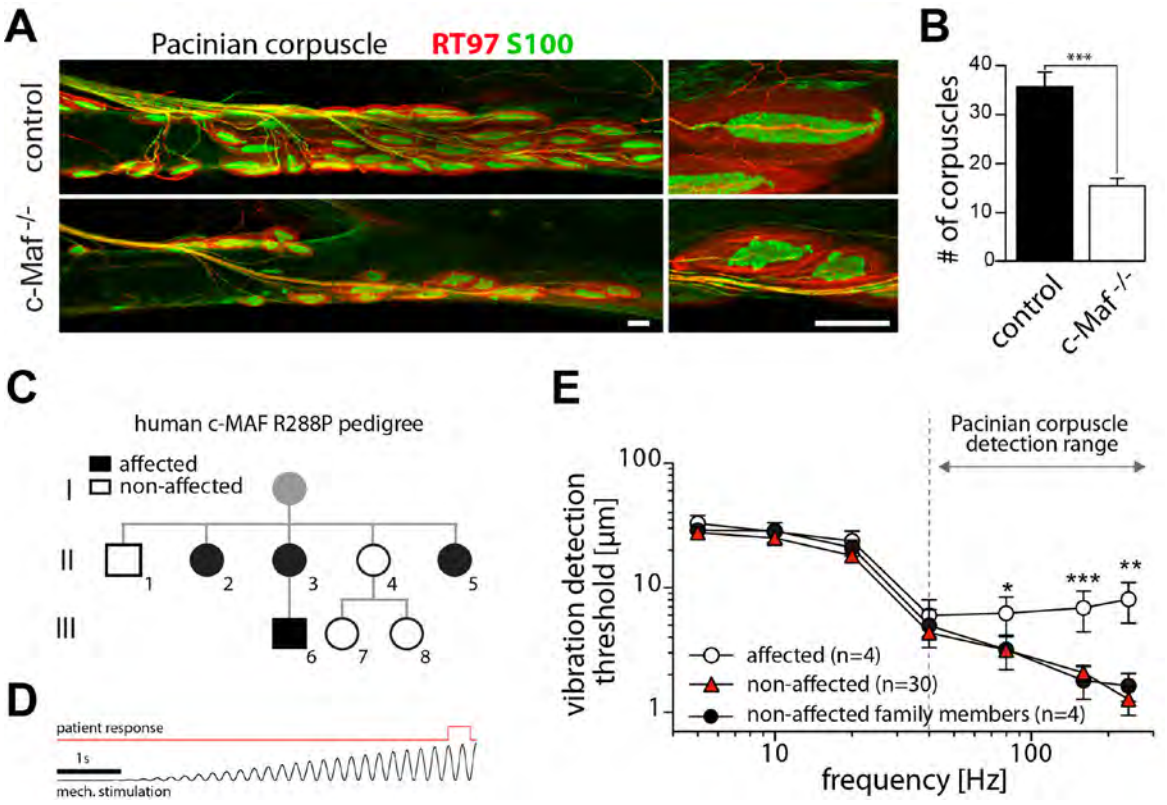


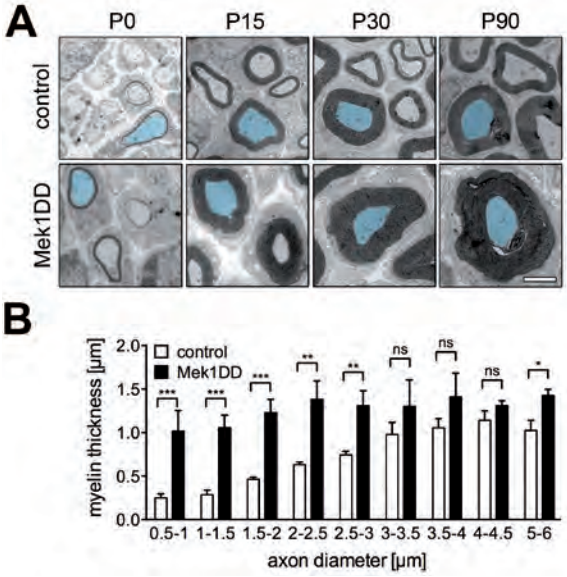
Fig. 2 c-Maf is required for the development of Pacinian corpuscles, the mechanosensory organs that detect high frequency vibration.
(A,B) c-Maf mutation in mice causes a loss of Pacinian corpuscles and disrupts the morphologies of remaining corpuscles. Pacinian corpuscles were visualized using RT97 and S100 antibodies that detect neurofilament 200 present in axons and S100 present in glial cells that surround the axons and form the end organ of the Pacinian corpuscle. (C) Pedigree of a family with the R288P c-MAF mutation; members carrying the mutant allele (black symbols) suffer from early-onset cataracts. (D,E) Detection threshold of vibrotactile stimuli. (D) Example of a vibration stimulus and response and (E) vibration detection threshold in control and four carriers of the R288P c-MAF mutation. In healthy participants and unaffected family members, the detection threshold decreases with increasing frequencies. The detection threshold of affected family members is aberrant at high but not low frequencies. Scale bars, 100 µm. Error bars, SEM.

The transcription factor c-Maf controls touch receptor development and function

Hagen Wende, Cyril Cheret, Maria E. Sheean, Katja Reuter, in collaboration with Stefan G. Lechner and Gary R. Lewin (Molecular Physiology, MDC), Patrick Carroll, Steeve Bourane, Alexandre Pattyn (NSERM U.1051, Montpellier) and Francis L. Munier (Jules Gonin Eye Hospital, Lausanne).

The sense of touch relies on detection of mechanical stimuli by specialized mechanosensory neurons. The scarcity of molecular data has made it difficult to analyze development of mechanoreceptors and to define the basis of their diversity and function. Physiologically, mechanoreceptors are classified as rapidly adapting, slowly adapting, and D-hair mechanoreceptors (RAMs, SAMs, and D-hairs). In addition, morphologically diverse end-organs are observed, and Pacinian corpuscles, Meissner corpuscles, and lanceolate endings (RAMs), as well as Merkel cell-neurite complexes and Ruffini corpuscles (SAMs) have been defined. In a screen for genes expressed in the developing murine nervous system, we found that the transcription factors c-Maf and MafA are co-expressed in mechanosensory neurons (RAMs).

We analyzed the role of c-Maf in mechanosensory neurons using mouse genetics. Pacinian corpuscles are the primary detectors of high-frequency vibration. In c-Maf mutants, the quantity of Pacinian corpuscles was reduced by more than 50%, and remaining corpuscles were small and irregularly shaped (Fig. 2A,B). In humans, dominant c-MAF mutations are associated with cataracts, but the effects of the mutation on mechanosensation had not been examined. Encouraged by our results in



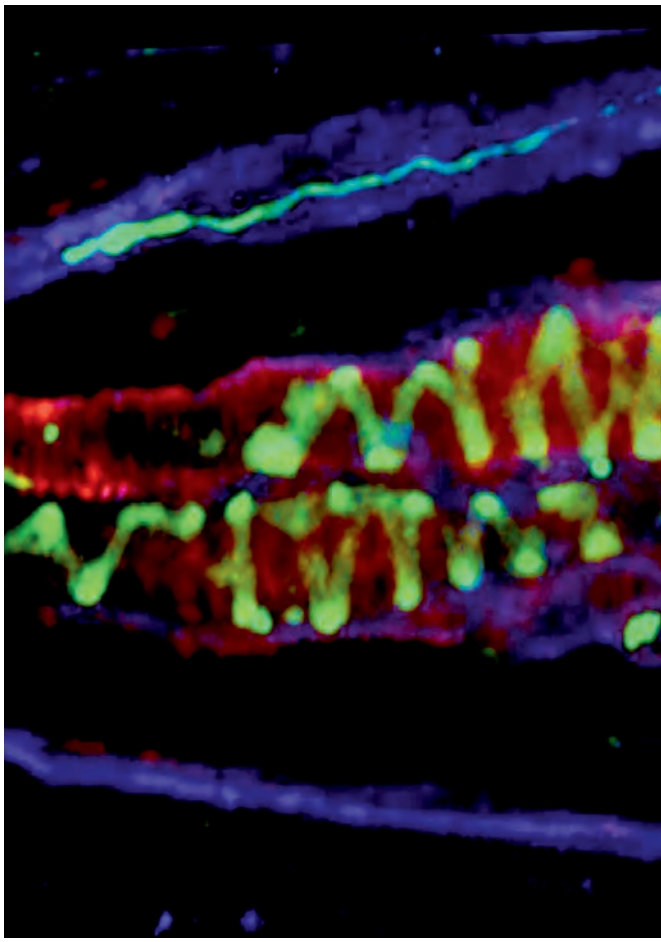


Fig. 3 Activation of MAPK overcomes the signals that end myelination. *(A) Sustained activation of MAPK by expression of Mek1DD in Schwann cells; expression of Mek1DD was initiated by the use of the cre/loxP technology at the onset of myelination. Displayed are nerves from control and Mek1DD mice at different ages (P0–P90) that were analyzed by electron microscopy (representative axons of similar diameters are false-colored in light blue). In control mice, myelin growth ends around P15, but in Mek1DD animals myelin grows continuously. (B) Quantification of myelin thickness in control and Mek1DD mice shows that hypermyelination of small diameter axons is particularly pronounced.*

mice, we tested touch sensitivity in humans that carry dominant c-MAF mutations, and observed that the mutation interferes with normal vibration detection (Fig. 2C-E). Thus, our analyses show that the transcription factor c-Maf/c-MAF is crucial for mechanosensory function in mice and humans (Wende et al., 2012).

Activation of MAPK overrides the termination of myelin growth and replaces Nrg1/ErbB3 signals during Schwann cell development and myelination

Maria E. Sheean, Cyril Cheret, Thomas Müller, in collaboration with Erik McShane and Matthias Selbach (Cell Signaling/Mass Spectrometry, MDC), Jan Walcher and Gary R. Lewin (Molecular Physiology, MDC), Annika Wulf-Goldenberg (Exp. Pharmacology and Oncology Berlin-Buch GmbH), Soraya Hoelper and Markus Krüger (MPI, Bad Nauheim), Alistair N. Garratt (Charité, Berlin), Dies Meijer (Erasmus University, Rotterdam), Klaus Rajewsky and Walter Birchmeier (Cancer Program, MDC).

Myelination depends on the synthesis of large amounts of myelin transcripts and proteins and is controlled by Nrg1/ErbB/Shp2 signaling. We developed a novel pulse labeling strategy based on stable, non -radioactive isotope labeling with amino acids in cell culture (SILAC) to measure the dynamics of myelin protein production in mice. This revealed that protein synthesis slows down in the maturing postnatal peripheral nervous system. Remarkably, sustained activation of MAPK signaling by expression of the Mek1DD allele overcomes the signals that end myelination, resulting in continuous myelin growth (Fig. 3A,B). MAPK activation is accompanied by minor changes in transcript levels and by a massive up-regulation of protein synthesis. Nrg1 and the ErbB2/3 receptors provide pivotal signals for Schwann cell development and myelination that are mediated by the tyrosine phosphatase Shp2. We found that activation of MAPK signaling by the Mek1DD allele releases Schwann cells from their natural ErbB3/Shp2 dependence. Nrg1/ErbB signals control different aspects of Schwann cell development, i.e. precursor proliferation and migration in early development, the onset of myelination, myelin growth and myelin protein production. Interestingly, activation of MAPK signaling by the Mek1DD allele largely rescues deficits in proliferation, migration, protein synthesis and myelination and thus suffices to replace Nrg1/ErbB/Shp2 signals in Schwann cells during early development and myelination (Sheean et al., 2014).

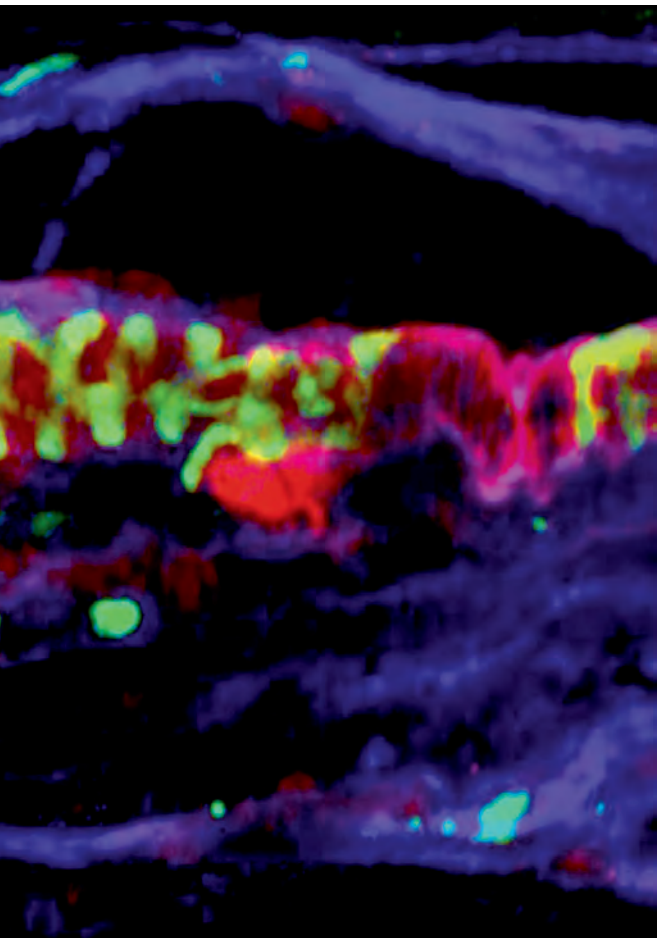


Fig. 4 Appearance of a muscle spindle, the proprioceptive organ of the muscle. *The spindle was visualized by immunohistology using antibodies against NF200 (green) that stain the proprioceptive sensory axon that wraps the muscle spindle and conveys information about muscle contraction, antibodies against Egr3 (red) that stain the spindle fiber, and antibodies against collagen IV (blue) that visualize the capsule surrounding the spindle.*

Bace1 and Neuregulin-1 cooperate to control formation and maintenance of muscle spindles

Cyril Cheret, Hagen Wende, in collaboration with Michael Willem, Sabina Tahirovic and Christian Haass (LMU and DZNE Munich), Florence R. Fricker and David L. Bennett (University of Oxford), Annika Wulf-Goldenberg (Experimental Pharmacology & Oncology Berlin-Buch GmbH), Klaus-Armin Nave (MPI Gottingen), Alistair N. Garratt (Charité Berlin) and Paul Saftig (Universität Kiel).

The protease β-secretase 1 (Bace1) was identified through its critical role in production of amyloid-β peptides (Aβ), the major component of amyloid plaques in Alzheimer's disease. Bace1 is considered a promising target for the treatment of this pathology, but processes additional substrates, among them Neuregulin-1 (Nrg1). Our biochemical analysis indicates that Bace1 processes the Ig-containing β1 Nrg1 (IgNrg1β1) isoform. Muscle spindles are specialized muscle fibers that detect muscle contraction and are innervated by proprioceptive sensory axons (Fig. 4). Muscle spindles are critical for motor coordination. We find that a graded reduction in IgNrg1 signal strength in mice results in increasingly severe deficits in formation and maturation of muscle spindles. Furthermore, we show that Bace1 is required for formation and maturation of the muscle spindle. Finally, pharmacological inhibition and conditional mutagenesis in adult animals demonstrate that Bace1 and Nrg1 are essential to sustain muscle spindles and to maintain motor coordination. Our results assign to Bace1 a role in the control of coordinated movement through its regulation of muscle spindle physiology, and implicate IgNrg1-dependent processing as a molecular mechanism (Cheret et al., 2013).

Selected Publications

RBP-J (Rbpsuh) is essential to maintain muscle progenitor cells and to generate satellite cells.Vasyutina E, Lenhard DC, Wende H, Erdmann B, Epstein JA, Birchmeier C. Proc Natl Acad Sci U S A. 2007;104(11):4443-8.

Colonization of the satellite cell niche by skeletal muscle progenitor cells depends on Notch signals.Bröhl D, Vasyutina E, Czajkowski MT, Griger J, Rassek C, Rahn HP, Purfürst B, Wende H, Birchmeier C. Dev Cell. 2012;23(3):469-81.

The transcription factor c-Maf controls touch receptor development and function. Wende H, Lechner SG, Cheret C, Bourane S, Kolanczyk ME, Pattyn A, Reuter K, Munier FL, Carroll P, Lewin GR, Birchmeier C. Science. 2012; 335(6074):1373-6.

Bace1 and Neuregulin-1 cooperate to control formation and maintenance of muscle spindles. Cheret C, Willem M, Fricker FR, Wende H, Wulf-Goldenberg A, Tahirovic S, Nave KA, Saftig P, Haass C, Garratt AN, Bennett DL, Birchmeier C. EMBO J. 2013; 32(14):2015-28.

Activation of MAPK overrides the termination of myelin growth and replaces Nrg1/ErbB3 signals during Schwann cell development and myelination. Sheean ME, McShane E, Cheret C, Walcher J, Müller T, Wulf-Goldenberg A, Hoelper S, Garratt AN, Krüger M, Rajewsky K, Meijer D, Birchmeier W, Lewin GR, Selbach M, Birchmeier C. Genes Dev. 2014; 28(3):290-303.

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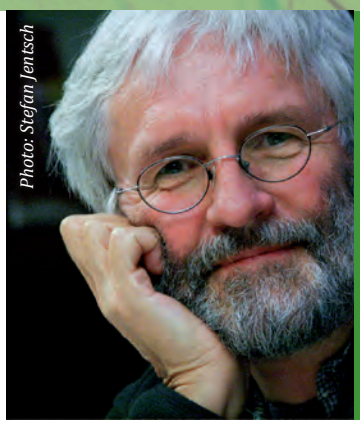
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Thomas J. Jentsch

Video:
How can tiny proteins in cell membranes prevent serious diseases?

Physiology and Pathology of Ion Transport

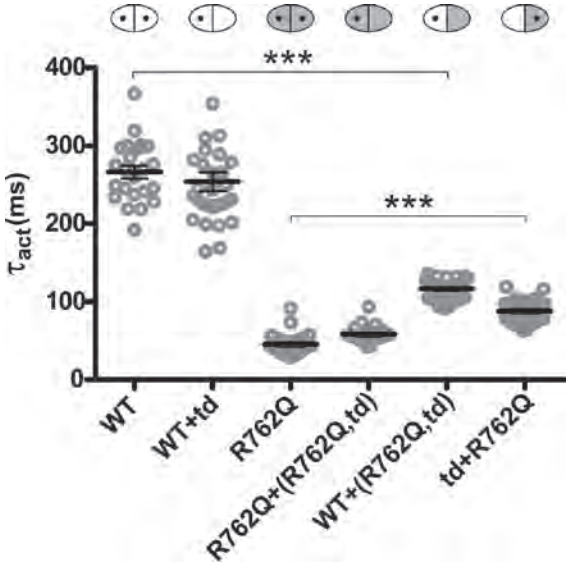
Ion transport across cellular membranes is crucial for cellular homeostasis and has integrative functions such as transepithelial transport or signal transduction. We study ion transport at various levels: from biophysical and structure-function analysis of transport proteins, their role in cellular functions – such as cell volume regulation or endocytosis – to the role in the organism. The physiological importance of ion transport proteins is often evident from pathologies resulting from their disruption in mice or men. We have discovered several human 'channelopathies' and have generated and analyzed many mouse models.
We focus on CLC chloride channels and transporters, Anoctamin Ca^{2+}-activated Cl^- channels, KCNQ potassium channels, and KCC potassium-chloride cotransporters. Their mutational inactivation led to pathologies ranging from epilepsy, deafness, and neurodegeneration to osteopetrosis and kidney stones. We are particularly interested in the control of neuronal excitability, sensory physiology and in the role of chloride and pH in endosomes and lysosomes.

(1) CLC chloride channels and transporters

Sabrina Jabs, Lilia Leisle, Carmen Ludwig, Tobias Stauber, Stefanie Weinert

The CLC gene family, discovered in our laboratory in 1990, encodes plasma membrane chloride channels and chloride transporters of intracellular membranes. By means of KO mouse models for most CLCs, in the past couple of years we identified associated β-subunits (barttin and Ostm1), discovered that certain vesicular CLCs are electrogenic Cl^-/H^+-exchangers, performed structure-function analysis, and uncovered several new pathologies resulting from their dysfunction. Vesicular CLCs were believed to be Cl^- channels that facilitate vesicular acidification by shunting proton pump currents, but surprisingly, most (or all) vesicular CLCs are Cl^-/H^+-exchangers. In 2010 we reported on mice in which ClC-5 and ClC-7 were converted with single point mutations to uncoupled, pure Cl^- channels. Surprisingly, these studies showed that these mice have largely the same phenotype as the corresponding KO lines, suggesting an important role of these transporters in vesicular Cl^- accumulation. However, some of the phenotypes of the 'uncoupled' $Clcn7^{unc/unc}$ mice were less severe than in $Clcn7^{-/-}$ mice, which may indicate a rescue of the lacking Cl^-/H^+-exchange by the Cl^- conductance, or ion transport-independent functions of the ClC-7 protein that is still present in $Clcn7^{unc/un}$ mice, but absent from $Clcn7^{-/-}$ mice. To distinguish between these possibilities, we generated a mouse with a point mutation that totally abolishes ion transport, but expresses normal amounts of correctly localized ClC-7. Analysis of these mice is in progress.

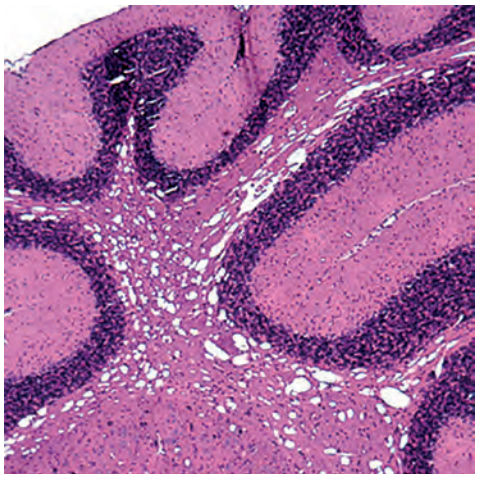
We also characterized functionally ClC-7 mutants identified in human patients and osteopetrotic cattle, and titrated the amount of osteoclast-expressed ClC-7 with respect to osteopetrosis in different transgenic mouse lines expressing ClC-7 in the background of $Clcn7^{-/-}$ mice. In biophysical studies we dissected further the slow voltage-dependent gating of ClC-7/Ostm1. It corresponds to the common gating known



Gating kinetics of ion flux through a ClC-7 monomer are influenced by the adjacent subunit of the dimer.
ClC-7 is a homodimeric lysosomal chloride transporter important for lysosomal function and bone degradation. Osteopetrosis and lysosomal storage disease are associated with accelerating mutations in the ClC-7 C terminus and the contacting intramembrane part.
We discovered that altered gating kinetics of one subunit affect the kinetics of the other subunit. Gating of ClC-7 involves both CLC subunits and requires non-covalent binding of cytoplasmic domains.
The graph shows individual and averaged time constants of activation (τ_{act}) of currents recorded from oocytes injected with RNA encoding WT ClC-7, the 'fast' mutant R762Q , a 1:4 ratio of WT and the transport deficient double mutant R762Q/td and a 1:4 co-injection of R762Q with the E314A (td) mutant. Note that the transport-deficient fast subunit accelerates gating in dimers with a WT subunit and that the transport-deficient WT subunit slows gating in dimers with a fast subunit, resulting in indistinguishable gating kinetics.
Symbols above diagrams are as follows: WT subunits are shown in white, subunits with an accelerating point mutation are in gray, and subunits carrying a transport-abolishing point mutation (td; E314A in ClC-7) are symbolized by a lack of the central "hole."
For details, see Ludwig C.F., Ullrich F., Leisle L., Stauber T., Jentsch T.J. (2013). Common gating of both CLC subunits underlies voltage-dependent activation of the $2Cl^-/H^+$-exchanger ClC-7/Ostm1. J. Biol. Chem. 288: 28611-28619.

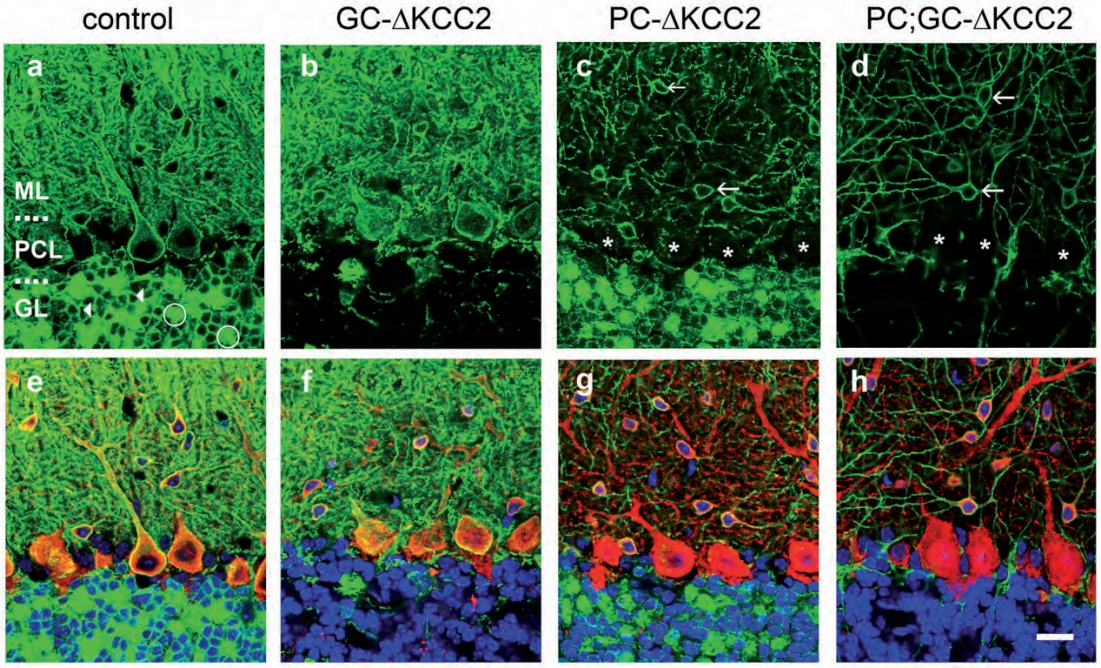
from CLC Cl^- channels and affects both pores in parallel, with a crucial role played by the cytoplasmic CBS domains that can bind non-covalently to the channel backbone when expressed for an independent cDNA.

We had previously shown that the disruption of ClC-2 in mice leads to leukodystrophy, and recently others identified CLCN2 mutations in a clinical subform of human leukodystrophy. In humans, megalencephalic leukoencephalopathy with subcortical cysts (MLC) can be caused by mutations in the multimembrane spanning protein



Myelin vacuolization in a Glialcam mouse model.
H&E stainings of sagittal paraffin section of cerebellum of a 52 weeks old GlialCAM$^{-/-}$ mouse. Novel mouse models deficient for the cell adhesion molecule, GlialCAM, and the membrane protein MLC1, manifest myelin vacuolization in cerebellar white matter tract. These two proteins in addition with ClC-2, a chloride channel, underlie forms of leukoencephalopathy, a pathology of the white matter, seen in humans. GlialCAM, MLC1, and ClC-2 in vivo form a protein complex at glial domains such as oligodendrocytes to regulate chloride transport.
For background information, see Jeworutzki E., López-Hernández T., Capdevila-Nortes X., Sirisi S., Bengtsson L., Montolio M., Zifarelli G., Arnedo T., Müller C.S., Schulte U., Nunes V., Martínez A., Jentsch T.J., Gasull X., Pusch M., Estévez R. (2012). GlialCAM, a protein defective in a leukodystrophy, serves as ClC-2 Cl^- channel auxiliary subunit. Neuron 73: 951-961.

MLC1, and in GlialCAM, an Ig-like cell adhesion molecule that physically interacts with MLC1. In a collaboration with R. Estévez (Barcelona) and M. Pusch (Genova) we showed that GlialCAM also interacts with ClC-2 and drastically changes its current properties. Moreover, GlialCAM directs both MLC1 and ClC-2 to cell-cell contacts of transfected cells. We now generated Glialcam$^{-/-}$ and Glialcam$^{dn/dn}$ mice, the latter carrying a mutation found in human leukodystrophy, and studied these mice in comparison with Mlc1$^{-/-}$ and Clcn2$^{-/-}$ mice. Immunohistochemstry indicated that GlialCAM is important for the localization of ClC-2 and MLC1 also in vivo, and that surprisingly the lack of MLC1 also affected the localization of ClC-2 and GlialCAM in both astrocytes and oligodendrocytes. ClC-2 currents were reduced in oligodendrocytes of all three mouse models, suggesting that the lack of glial chloride contributes to the pathology of all three forms of leukodystrophy. ClC-2 may be involved in extracellular ion homeostasis during neuronal activity. The observation that leukodystrophy was more severe in ClC-2/GlialCAM double KOs than in Clcn2$^{-/-}$ mice, however, indicates



Cell type-specific deletion of Kcc2 in the cerebellum.
*Progression of Kcc2 expression on Purkinje cells (PC) from $Kcc2^{lox/lox}$ (control) and PC-ΔKcc2 mice, averaged over all regions in the vermis. PCs showing somatic membrane staining were counted as positive cells (average±standard deviation, n=2, 1, 4, and 2 mice for P17, P21, P25, and P30, respectively (for each genotype); ~200 cells counted per animal). Also in granule cells of the vermis, Kcc2 deletion was complete at P25. (**a**–**h**) Immunofluorescent labelling for Kcc2 (green), parvalbumin (red), and nuclei (blue). (**e**–**h**) on sagittal sections of cerebellar cortex from adult mice (scale bar: 40 µm). (**a**, **e**) $Kcc2^{lox/lox}$ mice (control). (**b**, **f**) GC-ΔKcc2 mice with specific Kcc2 deletion in granule cells (GC). (**c**, **g**) PC-ΔKcc2 mice with specific deletion of Kcc2 in Purkinje cells. (**d**, **h**) PC;GC-ΔKcc2 mice with Kcc2 deletion in both PCs and GCs. Kcc2 expression remains in cerebellar interneurons (**c**, **d**, arrows). Asterisks in (**c**, **d**) indicate PC somata.*
Taken from Seja P., Schonewille M., Spitzmaul G., Badura A., Klein I., Rudhard Y., Wisden W., Hübner C.A., De Zeeuw C.I., Jentsch T.J. (2012). Raising cytosolic Cl^- in cerebellar granule cells affects their excitability and vestibulo-ocular learning. EMBO J. 31: 1217-1230.

that loss of GlialCAM has additional pathogenic effects. Conditional ClC-2 mice are in progress to pinpoint the cell type in which loss of ClC-2 is pathogenic.

(2) K-Cl^- cotransporters

Kathrin Gödde, Guillermo Spitzmaul, Patricia Seja

We have previously knocked-out all KCl-cotransporter isoforms (KCC1-4) in mice and have obtained specific and highly interesting phenotypes. We are continuing our studies on KCCs with conditional KOs. Our major focus is on KCCs expressed in neurons, where KCC2 in particular lowers the cytoplasmic chloride concentration. Such a low concentration is necessary for the inhibitory action of GABA and glycine, which act on ligand-gated chloride channels. In the reporting period we have been investigating various mouse lines in which KCC2 has been inactivated in specific sets of neurons.

We first focused on the cerebellum because of its rather simple architecture and the availability of Cre lines that allow specific gene deletions in Purkinje cells and granule cells. Patch-clamp analysis showed that Kcc2 was a major player in lowering cytoplasmic Cl^- in both types of neurons. Its deletion nearly abolished GABA-induced hyperpolarization of Purkinje cells, but in granule cells it merely affected excitability by membrane depolarization. Granule-specific deletion of Kcc2 from granule cells impaired consolidation of long-term phase learning in the vestibulo-ocular reflex, revealing a previously unknown role of these cells.

In another project that fits well to our studies on Ancotamin Cl^- channels and olfaction, we specifically deleted Kcc2 in mitral cells of the olfactory bulb in order to investigate the role of synaptic inhibition to odour discrimination. Electrophysiological and behavioural analysis of these mice is in progress.

(3) KCNQ potassium channels

Pawel Fidzinski, Matthias Heidenreich, Guillermo Spitzmaul

There are five different isoforms of KCNQ (Kv7) potassium channels, KCNQ1- KCNQ5. KCNQ2-KCNQ5 mediate 'M-currents' that regulate neuronal excitability. We had pre-

viously shown that KCNQ2 and KCNQ3 underlie a form of human epilepsy and that dominant KCNQ4 mutations are a cause of human deafness and have published a few years ago a mouse model for KCNQ4 deafness. In the last reporting period we showed that KCNQ4 also tunes rapidly adapting skin mechanoreceptor neurons to specific frequencies. We are currently investigating the role of other KCNQ isoforms in mechanosensation.

KCNQ4 is not only expressed in (outer) cochlear hair cells, but also in the vestibular organ where staining with KCNQ4 and KCNQ5 suggested that both channels are differentially expressed at the basolateral membrane of sensory hair cells. However, our investigation of the corresponding KO and KI mouse models now show that they are not expressed in hair cells, but in the adjacent large postsynaptic membrane of calyx synapses. Vestibulo-ocular reflexes showed moderate changes in the corresponding mouse models, with KCNQ4 playing the major role.

We also made major efforts to delineate the CNS function of KCNQ5, the least known member of this family. Our preliminary results, which include results from immunohistochemistry, slice electrophysiology, *in vivo* registrations and behavioural studies indicate that KCNQ5 plays a role in dampening synaptic inhibition, whereas KCNQ2 and 3 dampen excitation.

(4) Anoctamin (TMEM16) Ca^{2+}-activated Cl^- channels

Jonas Münch, Sebastian Albrecht, Gwendolyn Billig

We have started new projects to define functions of members of the newly identified Anoctamin family of Ca^{2+}-activated Cl^- channels. As a first result, we have shown that Ano2 is the long-sought Ca^{2+}-activated Cl^- channel of olfactory sensory neurons of the main olfactory epithelium (MOE), but that it is dispensable for olfaction. We are now turning our attention to the vomeronasal organ (VNO) that is responsible for the detection of pheromones and other cues like MHC peptides. The signal transduction cascade in the VNO is different from the MOE and their sensory neurons express both Ano1 and Ano2. We are generating mice lacking both Ano1 and Ano2 in olfactory sensory neurons and will cross them to mice lacking the upstream Trpc2 cation channel to genetically and functionally dissect the poorly understood signal transduction cascade in the VNO. In addition, we are working on other members of the Anoctamin family.

Selected Publications

Novarino G., Weinert S., Rickheit G., **Jentsch** T.J. (2010). Endosomal chloride-proton exchange rather than chloride conductance is crucial for renal endocytosis. Science 328: 1398-1401.

Weinert S., Jabs S., Supanchart C., Schweizer M., Gimber N., Richter M., Rademann J., Stauber T., Kornak U., **Jentsch** T.J. (2010). Lysosomal pathology and osteopetrosis upon loss of H^+-driven lysosomal Cl^- accumulation. Science 328:1401-1403.

Billig G.M., Pál B., Fidzinski P., **Jentsch** T.J. (2011). Ca^{2+}-activated Cl^--currents are dispensable for olfaction. Nature Neurosci. 14: 763-769.

Heidenreich M., Lechner S.G., Vardanyan V., Wetzel C., Cremers C.W., De Leenheer E.M., Aránguez G., Moreno-Pelayo M.A., **Jentsch** T.J.*, Lewin G.R.* (2012). KCNQ4 K^+ channels tune mechanoreceptors for normal touch sensation in mouse and man. Nature Neurosci. 15: 138-145.

Seja P., Schonewille M., Spitzmaul G., Badura A., Klein I., Rudhard Y., Wisden W., Hübner C.A., De Zeeuw C.I., **Jentsch** T.J. (2012). Raising cytosolic Cl^- in cerebellar granule cells affects their excitability and vestibulo-ocular learning. EMBO J. 31: 1217-1230.

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Photo: David Ausserhofer/MDC

Fritz G. Rathjen

Developmental Neurobiology

During embryonic development the guidance of axons and the precise recognition of targets are crucial for establishing the correct neuronal circuits in the brain. For the navigation to their target areas, growth cones of axons respond rapidly to guidance cues in their environment. Proper wiring of the brain is orchestrated by multiple molecular factors and cellular mechanisms including electrical activity. The research of Fritz G. Rathjen's group focuses on two major topics of the "wiring" problem: the branching of axons during outgrowth and modulation of electric activity by cell adhesion proteins which in turn might affect the formation of neuronal circuits.

Axonal branching allows nerve cells to communicate with distinct neuronal targets

The branching of axons is a morphological hallmark of virtually all neurons, and allows an individual nerve cell to innervate several distinct targets and to communicate with neurons in different parts of the brain. Axonal branching is therefore a key mechanism in the generation of neuronal circuits. It is crucial for normal brain function and impairments of branching might result in neurological disorders. In the last decades axonal branching has been therefore the subject of intense studies primarily by in vitro cultures, but our knowledge of the underlying molecular signaling mechanisms is still fragmentary.

A cGMP signaling cascade composed of CNP, Npr2 and cGKI is implicated in the bifurcation of DRG axons

Many neurons within the brain display extremely complex patterns of axonal arborization. In contrast, projections of dorsal root ganglion neurons (DRG) into the spinal cord display a stereotyped and simple pattern of axonal branching. The central projections of DRG neurons branch at three sites in a temporally controlled order: DRG axons enter the spinal cord and bifurcate into two arms resembling a 'T', and then extend in rostral and caudal directions. Roughly two days later, the two daughter axons generate collaterals which grow in a ventral direction to distinct laminae of the spinal cord where they branch a third time. Due to this simple pattern of branching the spinal cord represents an attractive system to identify factors implicated in axonal branching.

Previous studies by us using transgenic techniques showed that a cGMP-dependent signaling cascade composed of the ligand CNP, the receptor guanylyl cyclase Npr2 and the kinase cGKI is essential for axonal branching at the dorsal entry zone of the spinal cord. In the absence of any one of these components, DRG axons do not bifurcate but are able to turn either rostrally or caudally (Fig. 1). In contrast, collateral formation is not affected by this signaling system.

The cGMP signaling pathway that triggers bifurcation of DRG axons is also an essential regulator of the arborization of central afferents from cranial sensory ganglia

In the past years, we asked which neuronal populations apart from DRG neurons use this signaling system to regulate axonal branching. Using a transgenic technique to

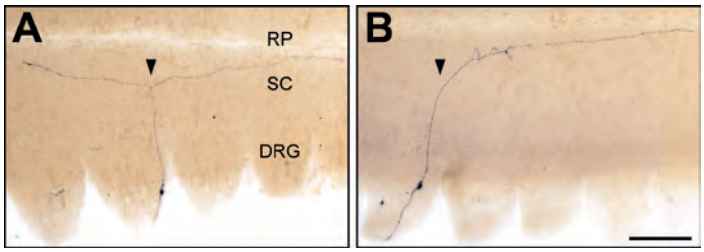


Figure 1: Loss of bifurcation of axons from DRG neurons in the absence of Npr2-mediated cGMP signaling. *Individual DRG neurons were labeled by a transgenic technique in wild-type mice (A) or in Npr2-mutant mice (B). The arrow heads point to a bifurcating and a non-bifurcating axon in the wild-type and Npr2-mutant mouse, respectively. Rostral is to the left. DRG, dorsal root ganglion; RP, roof plate; SC, spinal cord; scale bar, 250 µm.*

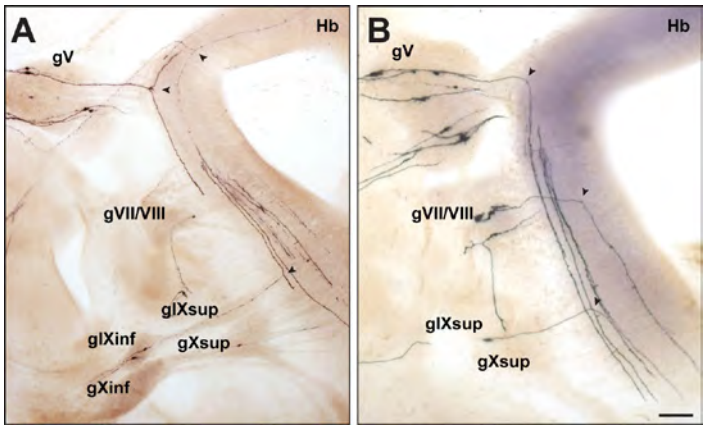


Figure 2: Bifurcation of axons from cranial sensory neurons depend on the receptor guanylyl cyclase Npr2. *Individual neurons from cranial sensory ganglia were labeled by a transgenic technique in wildtype (A) or in Npr2-mutant mice (B). The arrow heads point to bifurcating and non-bifurcating axons in the wild-type and Npr2 mutant mouse. gV, trigeminal ganglion; gVII, geniculate ganglion; gVIII, vestibular and spiral ganglion; gIX, glossopharyngeal ganglion; gX, vagus ganglion; sup, superior; inf, inferior; Hb, hindbrain; scale bar, 200 µm.*

label selectively a subset of Npr2-positive neurons, we demonstrated that the absence of Npr2 causes a complete loss of bifurcation of all cranial sensory axons, which instead establish only either ascending or descending projections (Fig. 2). Thus, axonal branching of cranial and dorsal root ganglia is regulated in an analogous manner by this cGMP signaling cascade. In contrast, collateral formation from the stem axons at the lateral margin of the hindbrain was not affected in the absence of cGMP – as also observed for DRG axons (Fig. 3).

Link between electric activity and cell adhesion molecules at developmental stages of the brain

In the mature brain electric activity is the language for communication between neurons. However, during embryonic and postnatal development electric activity of neurons is also implicated in shaping the structure of neuronal circuits of the brain. As soon as functional circuits forms, spontaneous activity in form of calcium waves becomes correlated between neighbouring neurons contributing to the refinements of established circuits. Cell adhesion molecules (CAM) might be considered as candidates to link electric activity of neurons to structural changes within neuronal circuits. CAMs establish initial cell-cell contacts and provide a platform for contact mediated intercellular signaling. Four structural groups of CAMs are expressed in the developing nervous system: cadherins/protocadherin; IgCAMs (immunoglobulin cell adhesion molecules), integrins and neurexins/neuroligins. Of these several IgCAMs are expressed at early stages and reveal a strong localization on axonal surfaces. We have therefore asked whether IgCAMs influence electric activity during development and tested whether the absence of an IgCAM affects calcium signalling. We observed that the IgCAM CAR specifically modulates intracellular calcium levels by regulating internal calcium stores. Our studies revealed an unexpected link between adhesion processes, calcium release and electric activity.

Further characterization indicated that CAR is a typical cell adhesion molecule that belongs to a specific subgroup of CAMs of the immunoglobulin superfamily. CAR is primarily expressed in the developing ner-

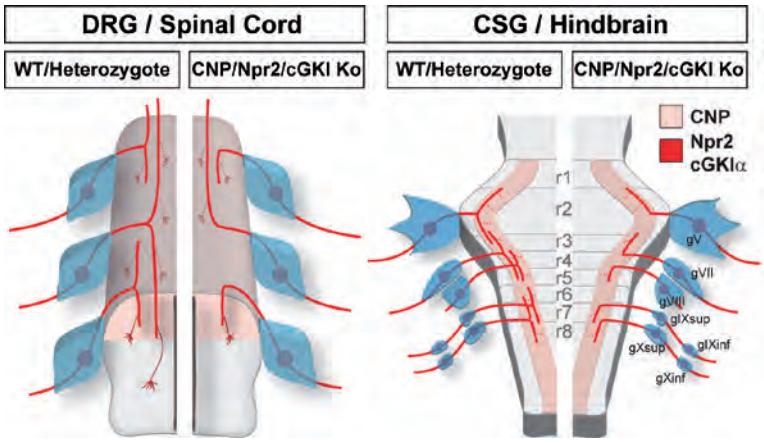


Figure 3: Schematic representation of the bifurcation defects observed in the absence of cGMP signaling in axons from dorsal root ganglia (DRG) in the spinal cord and cranial sensory ganglia (CSG) in the hindbrain. *The localization of Npr2 and cGKI or CNP in the axons, spinal cord or hindbrain is colored. The left part illustrates the wild-type, the right the mutant situation. r1-r8, rhombomeres 1-8.*

vous system and heart and is almost absent at mature stages. It reveals homophilic as well as heterophilic interactions. Our structural, binding as well as adhesion studies predict a flexible ectodomain of CAR allowing conformational shifts for cis or trans homophilic interactions on neurons.

Impaired presynaptic function and elimination of synapses in the absence of CALEB

CALEB, also termed neuroglycan C or CSPG5, is a neural protein composed of an N-terminal segment containing chondroitin sulfate chains followed by an acidic stretch, an EGF-like domain, a transmembrane and a cytoplasmic segment. CALEB expression is restricted to the central nervous system and appears to be generated as a precursor protein that becomes converted to a truncated transmembrane form with an exposed EGF domain and a secreted form. The conversion from a long form to a truncated form is promoted by electric activity. Our previous investigation on this protein showed an impaired synapse function in the absence of CALEB at developing but not mature stages.

In past years we studied the role of CALEB in the developing cerebellum which was motivated by our observation that CALEB-deficient mice displayed impaired motor coordination. Analysis of the neuronal connectivity of Purkinje cells by patch-clamp recordings demonstrated impairments of presynaptic maturation of inhibitory synapses. GABAergic synapses on Purkinje cells revealed decreased evoked amplitudes, altered paired-pulse facilitation and reduced depression after repetitive stimulation at early postnatal but not at mature stages.

Furthermore, the elimination of supernumerary climbing fiber synapses on Purkinje cells was found to be impaired in the absence of CALEB. For example, at postnatal day 8 in wild-type mice 54 percent of Purkinje cells have three or more climbing fiber synapses in contrast to mutants where this number is decreased to less than 25 percent (Fig. 4). The basic properties of the climbing fiber Purkinje cell synapse remained unaffected. The alterations observed by patch-clamp recordings correlated with a specific pattern and timing of expression of CALEB in Purkinje cells. It is dynamically regulated during development from a high chondroitinsulfate-containing form to a non-chondroitinsulfate-containing form. Thus, our results demonstrated an involvement of CALEB in presynaptic differentiation of cerebellar GABAergic synapses and revealed a role for CALEB in synapse elimination in Purkinje cells. These observations are restricted to early postnatal stages suggesting a transient role for CALEB in synapse maturation.

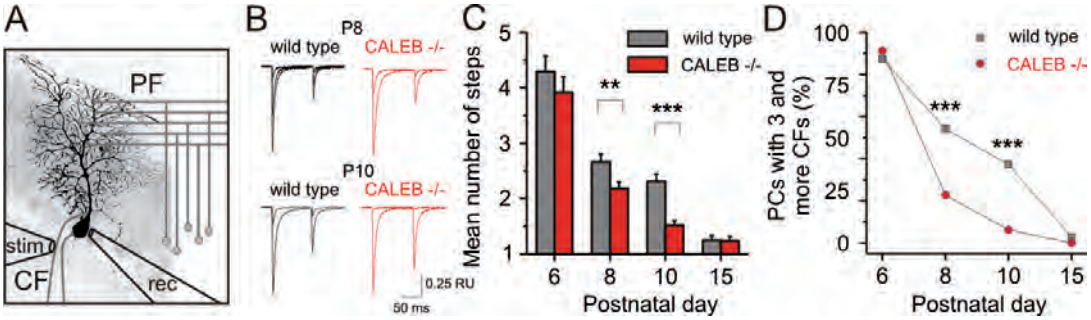


Figure 4: Elimination of climbing fiber-Purkinje cell synapses is impaired in the absence of CALEB. *The scheme (A) illustrates the electrophysiological design to estimate the number of climbing fiber synapses on Purkinje cells. Example traces (B) and mean number of steps (C), representing the mean number of synapses from wild-type and CALEB -/- Purkinje cells. D) The percentage of Purkinje cells with three and more climbing fiber synapses at different developmental stages in the absence of CALEB in comparison to wild-type Purkinje cells is shown. CF - climbing fiber; PF - parallel fiber; rec – recording pipette; stim – stimulation pipette.*

Selected Publications

Ter-Avetisyan, G., Rathjen, F.G. and Schmidt, H. (2014) Bifurcation of axons from cranial sensory neurons is disabled in the absence of Npr2-induced cGMP signaling. J. Neuroscience 34: 737-747.

Jüttner, R., Montag, D., Craveiro, R.B., Babich, A., Vetter, P. and Rathjen F.G. (2013) Impaired presynaptic function and elimination of synapses at premature stages during postnatal development of the cerebellum in the absence of CALEB (CSPG5/neurogly-can C). Europ. J. Neuroscience 38(9):3270-80.

Patzke, C., Max, K., Behlke, J., Schreiber, J., Schmidt, H., Dorner, A., Kröger, S., Henning, M., Otto, A., Heinemann, U. and Rathjen, F.G. (2010) The Coxsackie-Adenovirs receptor (CAR) reveals complex homophilic and heterophilic binding activities on neural cells. J. Neurosci. 30(8): 2897-2910

Schmidt, H., Stonkute, A., Jüttner, R., Kösling, D., Friebe, A. and Rathjen, F.G. (2009) C-type natriuretic peptide (CNP) is a bifurcation factor for sensory neurons. PNAS 106(39):16847-16854

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Photo: David Ausserhofer/MDC

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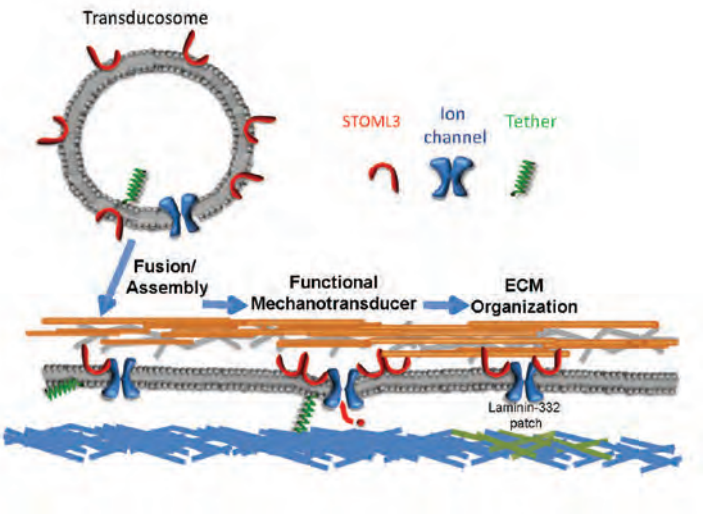
Molecular Physiology of Somatic Sensation

Somatic sensation includes all those sensations that we consciously feel after stimulation of the body, e.g. touch, warmth, cooling, or even limb movement. We experience these sensations as a direct result of the activation of sensory neurons that are located in the dorsal root ganglia (DRG). In our group we are interested in the molecular mechanisms that allow these neurons to transduce these varied stimuli. Sensory neurons can, for example, detect changes in temperature of the skin in non-noxious (not painful) as well as the noxious range (painful heat, or cold). They can also detect gentle movement of the skin as well as intense mechanical stimulation of the skin that is normally harmful. The nature of the transduction molecules involved together with the developmental events that lead to specification of the appropriate sensory neuron sub-types are actively investigated in the lab.

Molecular Basis of Mechanotransduction

Yinth Andrea Bernal-Sierra, Caglar Gök, Regina Herget, Liudmilla Lapatsina, Stefan G. Lechner, Mirko Moroni, Kate Poole, Christiane Wetzel

Mechanotransduction is the process whereby receptor proteins present in the endings of sensory neurons are able to detect mechanical stimulation of the tissue they innervate. We have used information from genetic experiments with the nematode worm *C.elegans* to identify possible vertebrate candidate proteins that might detect mechanical stimuli. Genetic screens for touch insensitive worms have turned up around 15 genes whose function is necessary to confer touch sensitivity. These genes were named mec for mechanically insensitive and we have focused on identifying a role mammalian orthologs of these genes in touch sensation. The mec genes in C.elegans have been proposed to work together in a mechanotransduction complex (Figure 1). An essential component of this complex is the membrane protein MEC-2 that forms a hairpin in the membrane and might regulate the activity of the mechanotransduction channels. We have cloned and characterized vertebrate homologues of mec genes and have created mouse mutant alleles to characterize the in vivo function of these genes. MEC-2 is a member of a large family of proteins that contain a stomatin-like domain. A member of this family called STOML3 (stomatin like protein-3) was cloned by our group, and we subsequently generated a mouse model with a null mutation of the STOML3 locus. In STOML3 mutant mice many mechanoreceptors (or touch receptors) in the skin do not work in the absence of the STOML3 protein. In order to analyze touch sensation in mice we also developed a novel behavioral assay



Model of the mechanotransduction complexes and how they may be inserted into the plasma membrane.

for touch driven behavior in rodents. This assay is based on the ability of mice to detect and react to gratings, which are fine enough to have a textured quality. STOML3 mutant mice have severe deficits in their ability to detect such textured surfaces.

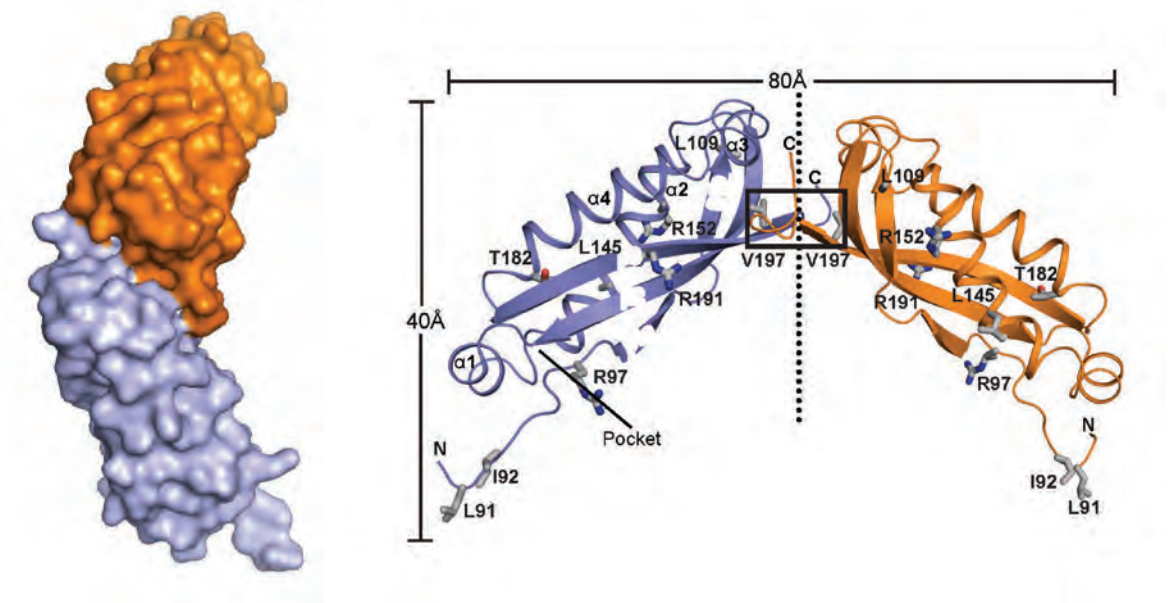
The central core of any mechanotransducer is an ion channel or channels(s), unfortunately the molecular nature of this channel has not yet been definitively identified in sensory neurons. There is some evidence that acid sensing ion channels (ASICs) which belong to the Deg/ENaC family of ion channels have an influence on the transduction of mechanical stimuli by sensory neurons. Indeed there is also evidence of genetic interactions between stomatin–domain proteins and acid sensing ion channels. Recently, two new mechanosensitive ion channels have been identified in mammals called Piezo1 and Piezo2. Using a newly developed assay based on elastomeric pili we could recently show that the sensitivity of Piezo ion channels to mechanical displacement is exquisitely tuned by the STOML3 protein (see Poole et al 2014). This work has been carried out in collaboration with Dr Kate Poole who is now a Cecille-Vogt fellow (see her pages in the research report).

Stomatin domain proteins, molecular membrane scaffolds with multiple roles in physiology and pathophysiology

Caglar Gök, James Hall, Liudmilla Lapatsina, Kate Poole, Jane Reznick

Stomatin-domain proteins can regulate both ion channels and transporter proteins. The structural and molecular basis of such regulation remained a mystery until recently. Together with the Daumke group we have used structural biology techniques and structure function studies to examine the molecular mechanisms of stomatin-domain protein function. By using X-ray crystallography techniques the structure of the mammalian stomatin protein was solved we showed that the basic building block of this protein is a banana-shaped dimer that can form the basis of higher order oligomeric structures in the membrane (Figure 2). The oligomerization of stomatin-domains appears to be necessary for their ability to modulate both ASIC proteins as well as mechanosensitive ion channels like Piezo1 and Piezo2 (Brand et al 2012; Poole et al 2014). Ongoing work in the lab seeks to examine the precise in vivo function of other poorly understood stomatin-domain containing proteins such as STOML1. The use of mouse models and advanced cellular imaging techniques as revealed an unexpected role for STOML1 in the metabolic disorders.

The activity of mechanosensitive ion channels expressed by sensory neurons can be measured using high-resolution electrophysiology techniques. We have recently shown that such ion channels in the membranes of cultured DRG neurons can be activated by stimuli in the nanometer range (Poole et al 2014). We have also gathered considerable evidence that the mechanosensitive channels are actually opened via a protein tether that attaches to laminin-containing extracellular matrices. In order to study the influence of different extracellular matrices on mechanotransduction and to quantify the tiny forces that are required to open mechanosensitive channels we have started to a use variety of new micro-fabrication techniques. For example, we have used micro-patterning of matrix molecules in order force neurons in culture to adopt morphologies that better match the in vivo situation. We can also use such patterning to test the local influence of specific matrix molecules on transduction ability or axon branching behavior. We have shown that sensory neurons can be made to grow to produce highly structured patterns in vivo (see Figure 1). Another application



Two stomatin domains from the stomatin protein form a dimeric interaction that gives rise to a banana-shaped protein (left). A high resolution structure of the stomatin-domain was obtained together with the Daumke group at the MDC (right) (see Brand et al 2012).

of micro-engineering is to make neurons grow on three dimensional surfaces that allow us to gauge the forces needed to open mechanosensitive ion channels in single cells (Poole et al 2014).

Touch, hearing and the development of mechanosensation

Henning Frenzel, Regina Herget, Stefan G. Lechner, Rabih Moshourab and Jan Walcher

Hereditary deafness is a relatively common phenomenon and a large number of genes have been identified that when mutated lead to deafness in mouse and man. We are working with several deaf mutant mice to examine whether genes required for normal mechanotransduction in the inner ear may also be required for normal cutaneous sensation. Our data indicate that members of the unconventional myosin protein family have a common function in sensory neurons and in hair cells, mechanotransducing cells of the inner ear. In both cell types these proteins may function to regulate the adaptation of the mechanotransduction channels. We are currently working on further hearing genes that may also affect cutaneous mechanosensation. The same genes as we study in the mouse are also mutated in humans and it is possible that the perception of cutaneous touch stimuli is altered in such patients. We have measured pyschometric functions in healthy and hearing impaired people in order to describe quantitatively differences in the perception of touch. We carried out a large twin study which showed that touch and also thermosensation are both heritable in humans. The results of these studies have also revealed that there appears to be a strong link between the sense of touch and the sense of hearing (Frenzel et al 2012). We could demonstrate that one gene, mutations in which, cause severe hearing loss and blindness is also associated with poor touch performance in humans. This gene USH2A encodes a large extracellular protein that is thought to form a structural link within the sterocillia of inner ear hair cells. The role of this protein in touch receptors remains to be investigated (Frenzel et al. 2012).

We have been interested in the development of mechanosensation for many years and it was remarkable how little was known in this area. We have recently shown, in a very detailed study, that sensory neurons acquire their competence to detect mechanical stimuli very early in embryonic development. Interestingly, very distinct developmental mechanisms are used to induce such competence in neurons that underlie touch sensation as opposed to nociception (Painful stimuli). For example, we have shown that NGF plays a critical role in the acquisition of transduction competence by nociceptors. The development of touch receptors is poorly understood but now in collaboration with Carmen Birchmeiers group it was found that one transcription factor c-Maf plays a decisive role in controlling the development of such receptors. Intriguingly, this factor is also necessary for the initiation of KCNQ4 expression in mechanoreceptors (Wende et al 2012).

The Naked Mole Rat an extremeophile mammal

Damir Omerbasic, Tania Kovalchuk, Jane Reznick, Valerie Begay-Müller

The naked mole rat is an unusual subterranean rodent in many respects. It is the only known poikilothermic mammal (ie. cold blooded), it lives in colonies with an insect-like social structure, and it is also the longest-lived rodent species known (lifetimes in excess of 25 yrs). Although this animal has normal acute pain responses it displays no hypersensitivity (so called hyperalgesia) to a variety of inflammatory and chemical stimuli. The naked mole rat completely lacks a neuronal or behavioral response to acid. We have shown that distinct gene variants encoding $Na_v1.7$ ion channels can account for the loss of acid sensitivity in this species. However, our interest in naked mole-rat physiology goes beyond the study of sensory systems. We are studying molecular changes in naked mole rats that may account for their complete lack of thermogenesis, and uniquely low metabolic rates.

Selected Publications

Brand J, Smith ESJ, Schwefel D, Lapatsina L, Poole K, Omerbašić D, Kozlenkov A, Behlke J, Lewin GR, Daumke O (2012) A stomatin dimer modulates the activity of acid-sensing ion channels. EMBO J 31:3635–3646.

Frenzel H, Bohlender J, Pinsker K, Wohlleben B, Tank J, Lechner SG, Schiska D, Jaijo T, Rüschendorf F, Saar K, Jordan J, Millán JM, Gross M, Lewin GR (2012) A genetic basis for mechanosensory traits in humans. PLoS Biol 10:e1001318.

Lapatsina L, Jira JA, Smith ESJ, Poole K, Kozlenkov A, Bilbao D, Lewin GR, Heppenstall PA (2012) Regulation of ASIC channels by a stomatin/STOML3 complex located in a mobile vesicle pool in sensory neurons. Open Biol 2:120096.

Poole K, Herget, R, Lapatsina L, Ngo HD, Lewin GR (2014) Tuning Piezo ion channels to detect molecular-scale movements relevant for fine touch. Nat Commun Mar 24;5:3520. doi: 10.1038/ncomms4520

Smith ESJ, Omerbašić D, Lechner SG, Anirudhan G, Lapatsina L, Lewin GR (2011) The molecular basis of acid insensitivity in the African naked mole-rat. Science 334:1557–1560.

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Photo: David Ausserhofer/MDC

Jochen Meier

RNA Editing and Hyperexcitability Disorders

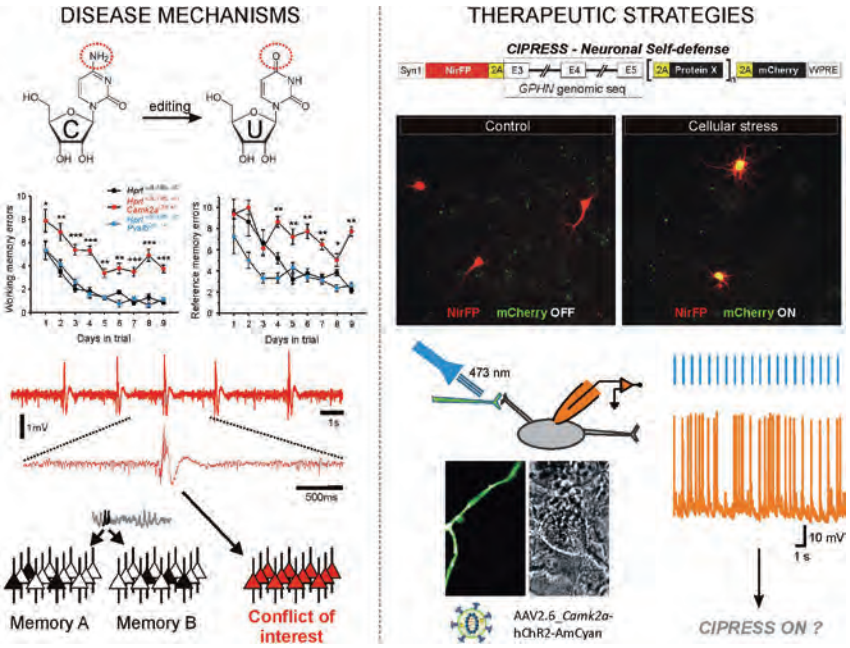
Excitatory and inhibitory transmission between neurons in the brain needs to be thoroughly regulated and coordinated. Deregulation of this coordination ultimately results in nervous system disorders. A core aspect of my work concerns the study of the brain at the molecular level, by investigating RNA editing and splicing. In particular, we analyze key components of the molecular machine responsible for inhibition of electrical impulses, i.e. glycine receptors (GlyR) and GABA type A receptors (GABA(A)R) as well as gephyrin. We elucidate the function of these molecules in physiological and pathophysiological processes on a molecular, cellular and systemic level.

Disease mechanisms

Epilepsy is a devastating neurodegenerative disease that severely deteriorates life quality due to unpredictable occurrence of seizures and associated cognitive dysfunction. Moreover, epilepsy patients suffer from severe psychiatric comorbidities including anxiety and depression. Most epilepsy syndromes have no discernable genetic component. This indicates that epileptogenesis is governed by disease-promoting molecular and cellular mechanisms of neuronal plasticity, which may vary from patient to patient, resulting in diverse clinical pictures of cryptogenic/idiopathic epilepsies. Therefore, new therapeutic strategies are needed to satisfy the variable demands of patients.

Prior to the development of effective individualized therapies, it must be elucidated whether a disease-associated mechanism represents either an adaptive form of plasticity that is able to compensate for the disease-causing insult, or a maladaptive form of plasticity that sustains disease progression. In a recent study, we identified a maladaptive form of neuronal plasticity by studying RNA editing of the neurotransmitter receptor for glycine (GlyR). The initial observation was that the expression of an RNA-edited GlyR variant is increased in hippocampi from patients with temporal lobe epilepsy (TLE) (Nat Neurosci 8:736, 2005; J Cell Mol Med 12:2848, 2008). We therefore generated a new animal model that allows the targeted and neuron type-specific expression of the RNA-edited GlyR variant. This model was then used to study cognitive function, learning and memory, and emotional behavior (J Clin Invest 124:696, 2014). The RNA-edited GlyR is specifically expressed at presynaptic terminals, and its presence increases the functional weight of such synapses in the hippocampal network. Thereby, homeostatic control of synaptic transmission and neural network excitability is changed, and the mice display symptoms that are reminiscent of the pathology of TLE. Notably, targeted expression of the RNA-edited GlyR in excitatory glutamatergic neurons provoked seizure-like activity and cognitive dysfunction, and expression in inhibitory synapses of parvalbumin-positive interneurons resulted in anxiety. Thus, the same molecule triggered distinct psychopathological symptoms of TLE. This study identified a maladaptive and disease-causing mechanism of neuronal plasticity that will serve as a good starting point for the development of an individualized treatment. We are expanding these experiments to target the expression of the RNA-edited GlyR variant to other neuronal types and neurotransmitter systems. Furthermore, we are applying advanced imaging techniques using molecular beacons to gain insights into the neuron type-specific regulation of RNA editing.

Gephyrin is a postsynaptic scaffold protein required for stabilization of GlyR and GABA(A)R at inhibitory synapses (Nat Neurosci 4:253, 2001; Eur J Neurosci 37:544, 2013). Consider-



DISEASE MECHANISMS: *Deamination commutes cytidine to uridine. C-to-U RNA editing produces gain-of-function GlyR and is increased in patients with TLE. In a corresponding knock-in mouse model, expression of the GlyR RNA variant in glutamatergic principal neurons impaired cognitive function and memory formation. Altered gamma frequency network oscillatory activity due to spontaneous recurrent epileptiform network activity and resulting conflict of interest of neurons during processing of sensory context can explain the animal phenotype (J Clin Invest 124:696, 2014).*
THERAPEUTIC STRATEGIES: *The new molecular tool 'CIPRESS' identifies stress-susceptible neurons (Brain 133:3778, 2010) and effectuates neuronal self-defense through expression of selected proteins (protein X). Using optogenetics and electrical stimulation of the trisynaptic hippocampal loop, it will also help identifying neuronal activity patterns that cause cellular stress and induce protein expression.*

able functional heterogeneity of gephyrin arises from alternative mRNA splicing (Mol Cell Neurosci 16:566, 2000; J Neurosci 24:1398, 2004). We identified gephyrin splice variants that are specifically expressed in glial or neuronal cells (J Biol Chem 283:17370, 2008), indicating that gephyrin RNA splicing is regulated in a cell type-specific way. Recently, we isolated from hippocampi of TLE patients irregularly spliced gephyrin RNA variants. These variants lack several exons and encode neuronal gephyrins with dominant-negative activities that de-stabilize postsynaptic receptors and weaken transmission at inhibitory synapses. We discovered that neuronal activity or calcium-dependent cellular stress induces the skipping of exons in the gephyrin-coding mRNA, leading to frameshift and premature termination of protein synthesis (Brain 133:3778, 2010). Thus, changes in RNA processing of GlyR-coding mRNA and impaired splicing of the gephyrin-coding mRNA may cooperate in the vicious circle of pathogenic mechanisms that impair neural network homeostasis and sustain the disease progression.

Therapeutic strategies

Several possibilities exist that can interrupt the vicious circle of impaired plasticity and disease progression. First, we are performing drug screening to identify specific antagonists of the pathogenic GlyR variant produced by RNA editing. The identified compounds will be validated in the mouse model described above. Second, based on the finding that exon skipping in gephyrin-coding mRNA induces frameshift in the protein coding sequence, we developed a new molecular tool for neuronal self-defense (cf. 'CIPRESS' project; www.neuron-eranet.eu/en/317.php). This system permits induction of protein expression in response to cellular stress that accompanies epileptogenesis. We are asking which candidate proteins (or combinations) can suppress seizure activity in vivo by using in utero electroporation of different expression constructs, or by lentiviral expression in slice culture of the human epileptic hippocampus. We will also determine the characteristics of the network activity that causes cellular stress and candidate gene expression using optogenetics. This work should allow the characterization of disease causing mechanisms of plasticity, for instance changes in the regulation of neuronal pH and chloride, energy metabolism, calcium-dependent proteolysis, and inhibitory actions of GABA.

Selected Publications

A.Winkelmann, N.Maggio, J.Eller, G.Caliskan, M.Semtner, U.Häussler, R.Jüttner, T.Dugladze, B.Smolinsky, S.Kowalczyk, E.Chronowska, G.Schwarz, F.G.Rathjen, G.Rechavi, C.A.Haas, A.Kulik, T.Gloveli, U.Heinemann, J.C.Meier (2014). Changes in neural network homeostasis trigger neuropsychiatric symptoms. **Journal of Clinical Investigation** 124:696-711.

T.Dugladze, N.Maziashvili, C.Börgers, S.Gurgenidze, U.Häussler, A.Winkelmann, C.A.Haas, J.C.Meier, I.Vida, N.Kopell, T.Gloveli (2013). $GABA_B$ autoreceptor-mediated cell-type specific reduction of inhibition in epileptic mice. **Proceedings of the National Academy of Sciences** 110:15073-15078.

B. Förstera, A.A.Belaidi, R.Jüttner, C.Bernert, M.Tsokos, T.-N.Lehmann, P.Horn, G.Schwarz, J.C.Meier (2010). Irregular RNA splicing curtails postsynaptic gephyrin in the cornu ammonis of patients with epilepsy. **Brain** 133: 3778-3794.

J.C. Meier*, C.Henneberger, I.Melnick, C.Racca, R.J.Harvey, U.Heinemann, V.Schmieden, R.Grantyn (2005). RNA editing produces P185L amino acid substitution in glycine receptor a3 resulting in high agonist potency. **Nature Neuroscience** 8:736-744.

J. Meier, C.Vannier, A.Sergé, A.Triller, D.Choquet (2001). Fast and reversible trapping of surface glycine receptors by gephyrin. **Nature Neuroscience** 4:253-260.

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Photo: David Ausserhofer/MDC

Björn Christian Schroeder

Signaling and Transport Processes

Our work focuses on signal and transport processes involving members of the recently identified TMEM16 family of membrane proteins. Mutations in several TMEM16 genes cause human inherited diseases including muscular dystrophy, the rare bleeding disorder Scott syndrome, and variants of cerebellar ataxia and dystonia. So far two of the TMEM16 proteins have been shown to mediate calcium activated chloride currents (CaCCs), known to be important for various functions including photo transduction, pain perception, and smooth muscle contraction. It is still unknown whether other members of this family are ion transport proteins and how they operate within cells and whole organisms. Using cultured cells and transgenic animals, we try to better understand the function of TMEM16 proteins under physiological and pathophysiological conditions.

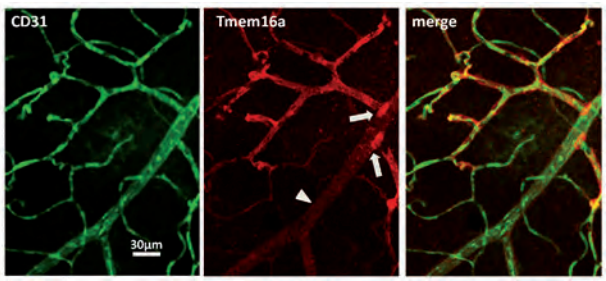
Calcium activated chloride channels in blood pressure regulation

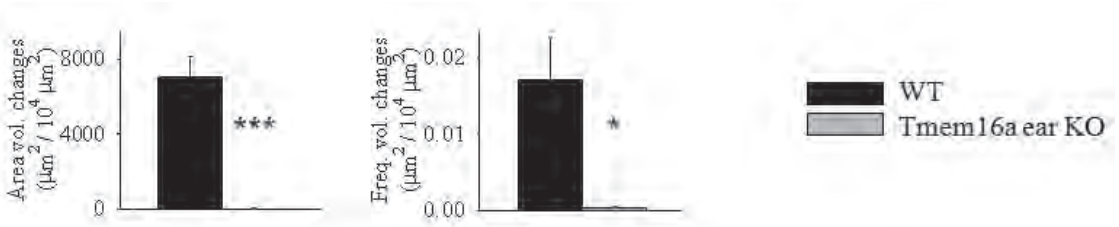
High blood pressure is the leading risk factor for death worldwide. One of the hallmarks is a rise of peripheral vascular resistance, which largely depends on the tone of arterioles. We generated a vascular smooth muscle cell specific Tmem16a knock out mouse to test if CaCCs are involved in the regulation of systemic arterial blood pressure. Disruption of the calcium activated chloride channel Tmem16a in vascular cells resulted in decreased systemic blood pressure, as well as a decrease in angiotensin II-induced hypertension in mice. Analysis of calcium activated chloride currents (CaCCs) along the vascular tree of wild-type mice identified substantial CaCCs in vascular smooth muscle cells (VSMCs) of the aorta and carotid arteries. CaCCs were small or even absent in medium sized vessels like mesenteric arteries or larger retinal arterioles. In small vessels, which are responsible for about 80% of the total vascular resistance, where VSMCs are gradually replaced by contractile pericytes, CaCCs were particularly large in wild-type but absent in Tmem16a mutant mice. Expression analysis for Tmem16a along the vascular tree showed a similar distribution (Figure 1). Out data suggest that Tmem16a plays a general role in arteriolar and capillary blood flow and is a promising target for the treatment of hypertension.

The role of Tmem16a in hearing

Inner supporting cells in Kölliker's organ, a developmentally transient structure within the organ of Corti, have been shown to initiate calcium waves and suggested to be the origin of the spontaneous activity. Cellular crenation events with unknown function occur during these waves.

Chloride channel Tmem16a is expressed in small, but not in large arterioles. *Retinal whole-mount, double-immunostainings for the endothelial cell marker CD31 (left), which is expressed in arterial and capillary endothelial cells, and Tmem16a (middle) showing a large Tmem16a-negative primary arteriole (arrow head) and second and higher-order arterioles branching from it, and overlay (right). Arrows indicate strongly-labeled cells at branching points of second-order arterioles.*





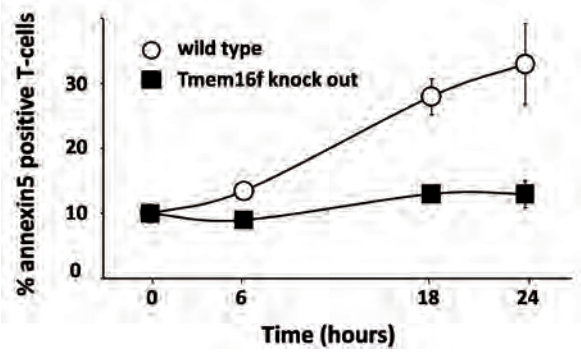
Tmem16a expression is necessary for the generation of volume changes in Kölliker's organ.
Spontaneous epithelial cell volume changes in acutely isolated cochlea devoid of Tmem16a of P5-P7 animals are almost absent. Compared to wild-type, typical event sizes are very small (left) and rare (right).

Because chloride channels have been implicated in cellular volume regulation, we investigated whether Tmem16a is involved in this process. We found high Tmem16a expression in Kölliker's organ (Figure 2). Disruption of Tmem16a under the control of the Pax2 promoter resulted in the complete absence of crenation events, identifying Tmem16a as key mediator for these volume changes. Moreover calcium waves in Kölliker's organ were reduced to small calcium puffs in knockout mice. This might be explained by the absence of mechanical stress, which facilitates ATP release of epithelial cells during activation and is required for the formation of calcium waves. The concept of chloride channels being important for the generation of calcium waves is new and has never been proposed. It is likely that Tmem16a plays a similar role for calcium waves in other organs like airway epithelial cells.

Molecular functions of Tmem16f in the immune system

Inner and outer leaflet of the cellular plasma membrane have different lipid compositions.

Diminished phosphatidylserine exposure of Tmem16f-deficient T-cells after activation.
T-cells isolated from spleen of wild-type and Tmem16f knockout mice were stimulated with anti-CD3 + anti-CD28 and assayed for surface phosphatidylserine using an Annexin V binding assay at the time indicated. The increase in surface expression of phosphatidylserine found in wild-type cells is absent in mutant T-cells.



Especially the negatively charged phospholipid phosphatidylserine is normally located in the inner but not the outer leaflet. During activation of platelets phosphatidylserine is redistributed to the outer leaflet where it provides a binding side for interacting coagulation factors. In Scott syndrome, a bleeding disorder caused by mutations in the TMEM16F gene, this translocation is disturbed, resulting in impaired blood clotting, and changed morphology of activated platelets.

We generated Tmem16f deficient mice to investigate the role of Tmem16f in other cells, especially of the immune system. B-cells and T-cells isolated from Tmem16f knock-out mice showed a strong diminished phosphatidylserine exposure after stimulation (Figure 3). In addition we found a reduced secretion of cytokines, and microparticle shedding from Tmem16f deficient cells from knockout mice, which suggests that Scott patients also have an altered immune response.

We hope that our research will not only help to understand the function of various TMEM16 genes, but also to prevent diseases and conditions like hypertension, stroke or autoimmune disorders.

Selected Publications

Heinze C, Seniuk A, Sokolov MV, Huebner AK, Klementowicz AE, Szijártó IA, Schleifenbaum J, Vitzthum H, Gollasch M, Ehmke H, Schroeder BC, Hübner CA. (2014). Disruption of vascular Ca2+-activated chloride currents lowers blood pressure. J Clin Invest. 124, 675-86.

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Photo: David Ausserhofer/MDC

James Poulet

Neural Circuits and Behaviour

We study sensory processing and perception in vivo to understand healthy brain function. We work on a relevant sensory system in a genetically tractable model animal - the mouse forepaw somatosensory system. In awake, behaving animals, sensory processing involves the integration of external and internally generated signals in the brain to generate a single coherent percept. We are especially interested in synaptic and cellular mechanisms underlying "sensory integration" in neocortex - an area of the brain that can integrate across sensory modalities and is under profound internal control by distinct brain states. To address these questions we use a combination of techniques including two-photon microscopy, optogenetics, electrophysiology (especially whole-cell patch clamp) and behavioural training.

The mouse forepaw system

The mouse forepaw somatosensory system has a similar structure and function to the human hand (Figure 1). Mice use their forepaws not only for locomotion but also for manipulating and grasping objects and sensing their immediate environment. The mouse forepaw has 5 digits, albeit the first more of a stump than a thumb. The glabrous skin surface is innervated with a full complement of primary sensory afferent neurons that process light touch, proprioception, deep touch, temperature and pain. Somatosensation is therefore a multimodal, integrative sense. (See Figure 1)

Synaptic mechanisms of somatosensory integration

In vivo, cortical neurons integrate thousands of synaptic inputs to generate action potential outputs during different brain states. How brain states affect synaptic integration is debated as almost nothing is known about the properties of monosynaptic transmission *in vivo.* We developed triple two-photon targeted whole-cell patch clamp recordings and optogenetic techniques to allow simultaneous recordings of synaptically connected neurons in vivo. This work provides new approaches to investigate active synapses in vivo and will allow us to target molecular mechanisms underlying state-dependent synaptic integration between genetically identified cell types *in vivo*.

Somatosensory processing by subtypes of cortical neuron

Neighbouring layer 2/3 cortical excitatory neurons show considerable heterogeneity in their responses to sensory stimulation. One explanation is that different subtypes of cortical pyramidal neurons exist with different sensory response properties in layer 2/3, but there are no molecular markers available to investigate this hypothesis. In this project we use the fosGFP mouse to investigate a subset of layer 2 excitatory neurons in mouse somatosensory cortex that are distinguished by expression of the activity-dependent fosGFP reporter gene under basal conditions. We showed that fosGFP+ neurons have higher spontaneous firing rates in vivo than fosGFP- pyramidal neurons (Yassin et al., 2010). Recent two-photon targeted dual whole-cell recordings in combination with optogenetic and sensory stimulation have shown that

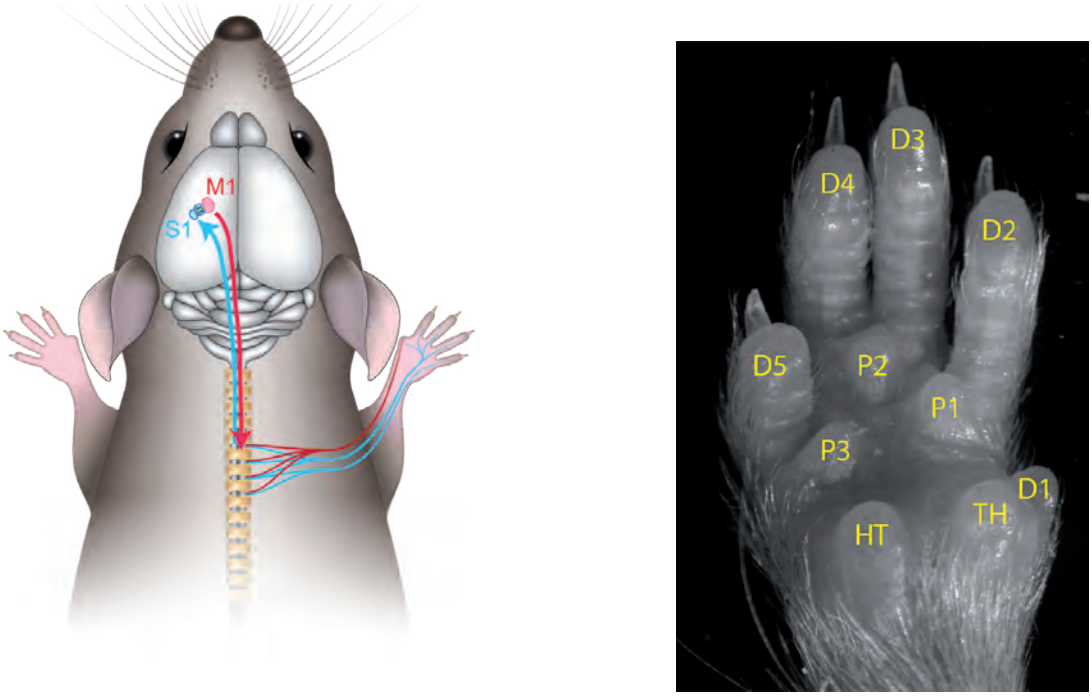


***Figure 1.* The mouse forepaw system. A,** *a cartoon of the mouse forepaw system. Blue represents the sensory pathway from paw via spinal chord and thalamus to primary somatosensory cortex (S1), red highlights the motor pathway from motor cortex (M1) to spinal cord and forelimb that can drive paw movement.* **B,** *image of the glabrous skin of the right forepaw, mice have 5 digits (D1-D5) and 5 pads (P1-3, Thenar pad, TH, and the HypoThenar pad, HT).*

fosGFP+ve neurons also have distinct sensory response properties and thalamocortical wiring.

Somatosensory perception

The temperature of an object provides important information for animals performing tactile tasks. Temperature and touch input are thought to closely interact in the brain but the primary sensory afferents in the skin and central circuits they engage to enable the perception of surface temperature are not clear. Here we have developed training routines for mice to report thermal and tactile cues delivered to the forepaw with high accuracy. We found that the forepaw primary somatosensory cortex, a region normally associated with tactile perception, mediates mild cooling processing and perception. In collaboration with the Lewin group at the MDC, we show that sensory afferent fibres in the skin, that are normally thought of as pain receptors, are likely candidates for forwarding mild cold information to the central nervous system. This project has established a new genetically tractable model system to study thermal perception and will allow us to examine how multimodal sensory inputs are integrated and perceived – a fundamental yet poorly understood function of neocortex.

Selected Publications

Barth A., and Poulet J.F.A. (2012) Experimental evidence for sparse cortical coding. Trends Neurosci. 35:345-55

Poulet J.F.A*., Fernandez, L*. Crochet S.* and Petersen C.C. (2012) Thalamic control of cortical brain states. Nature Neuroscience. 15:370-372 *equal contribution

Maier N.*, Tejero-Cantero A.*, Dorrn A, Winterer J., Beed P, Morris G., Kempter R., Poulet J.F.A.*, Leibold C.* and Schmitz D.* (2011) Coherent phasic excitation during hippocampal ripples. Neuron. 2011 72:137-52. *equal contribution

Yassin L, Benedetti B.L., Jouhanneau J-S., Wen J.A., Poulet J.F.A., Barth A.L., An embedded subnetwork of highly active neurons in the neocortex. (2010) Neuron 68: 1043-50.

Poulet J.F.A. and Petersen C. C. (2008) Internal brain state regulates membrane potential synchrony in barrel cortex of behaving mice. Nature, 454: 881-885.

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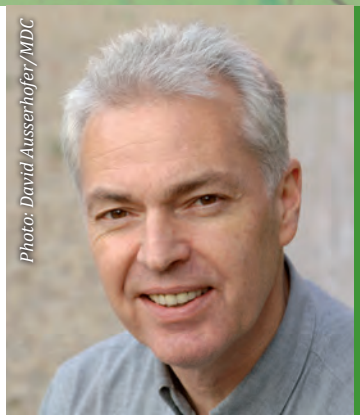


Photo: David Ausserhofer/MDC

Helmut Kettenmann

Cellular Neurosciences

Our goal is to understand the role of glial cells in physiology and pathology. We focus on questions how glial cells communicate among each other. We study the expression of transmitter receptors in microglial cells and how activation of these receptors influences microglial functions. This is of particular interest within the context of pathology and we are currently studying this question in stroke, glioma, Alzheimer's disease and multiple sclerosis. A fourth line of research addresses the question as to how glioma cells interact with the intrinsic brain cells, specifically microglia and astrocytes. We are aiming to understand this interaction on a molecular level, in particular with the hope of identifying therapeutic targets.

Introduction

The central nervous system contains two major cell populations, neurons and glial cells. The neurons are regarded as the elements mediating the electrical activity in the brain. As a consequence, neuroscience research of the past has focused on this cell type. The functional role of glial cells is not as obvious: while they were first described as cells providing only structural support to neurons, a series of more recent studies on glial cell function has attracted the attention of the neuroscience community. It has become evident that glial cells are essential for the proper functioning of the brain. The different types of glial cells fulfil distinct tasks. Oligodendrocytes are the myelin-forming cells of the central nervous system and ensure a rapid signal conduction in the white matter. The role of astrocytes is less well defined; they provide guiding structures during development and represent important elements for controlling the composition of the extracellular space mediating signals between the brain endothelium and neurons. They form intimate contact with synapses and neuronal activity results in astrocyte responses. Microglial cells are immune-competent cells in the brain and their functional role is best defined as the first responsive elements during pathologic events. The present research program is focused on four topics: (1) the extent of gap junction coupling within the glial network and its impact on brain function (2) the functional expression of transmitter receptors in microglia (3) the response of microglial cells to brain injury and (4) the interaction of gliomas with microglia and astrocytes.

Structural and functional analyses of panglial coupling networks and its impact on brain signaling

Nadine Richter, Christiane Nolte (in collaboration with Christian Steinhäuser, University of Bonn)

Gap junction coupling among glial cells plays an important role for brain homeostasis, brain communication and brain energy supply. Most previous studies have focused on the network formed by astrocytes. We now characterize communication between all types of glial cells, including communication of astrocytes with oligodendrocytes, with the newly described class of glial cells, the NG2 cells, and with microglia. We address the question whether these panglial networks are heterogeneous with respect to brain region, comparing different white and gray matter regions. We will characterize junctional properties and their modulation by neurotransmitters. We will also address the question whether the coupled network has functional implications for energy supply to neurons. We aim to present

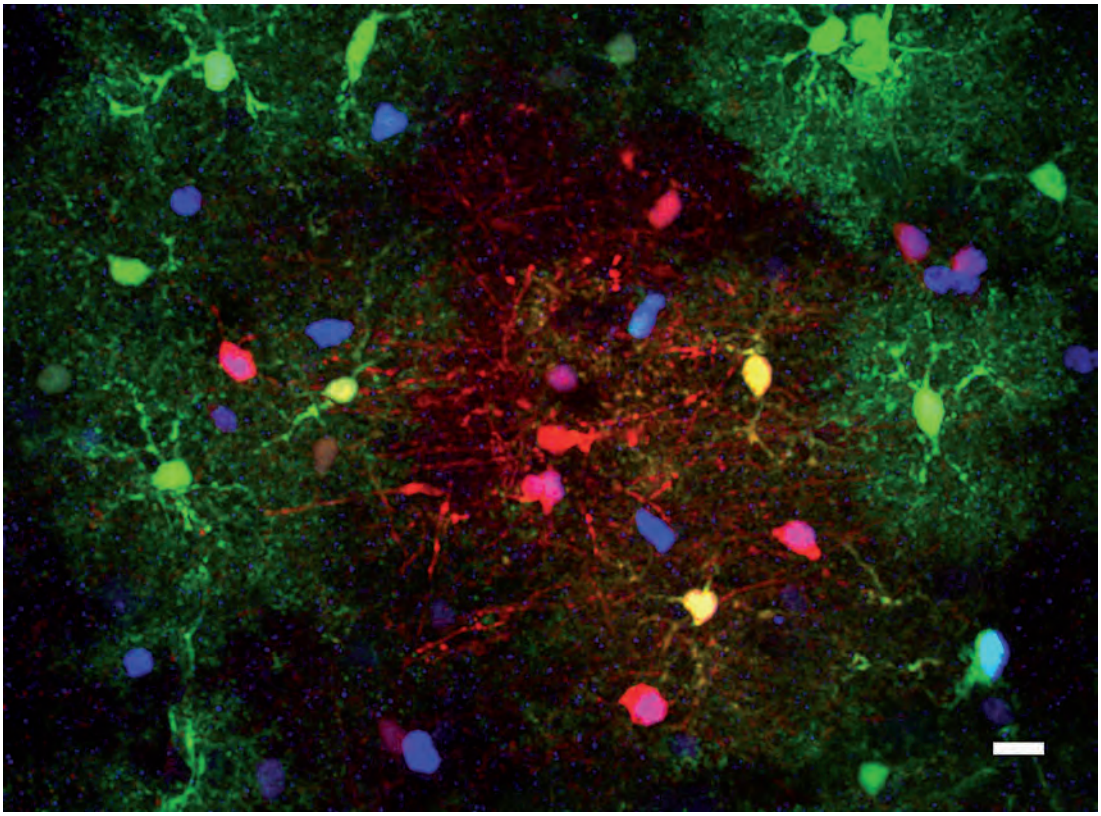


Figure 1: *The confocal image shows a biocytin filled panglial network in the cortex of a GFAP/eGFP mouse. In this mouse astrocytes express the enhanced green fluorescent protein under the GFAP (glial fibrillary acidic protein) promoter. A single astrocyte was injected with the gap junction permeable tracer biocytin via the whole-cell patch clamp technique. The biocytin is subsequently labeled with streptavidin Cy3 (red) while the oligodendrocytes (blue) in the network are immunolabeled with the marker Olig2. Apparently in this panglial network some astrocytes also express Olig2 (e.g. the green and blue fluorescent astocytes in the bottom right corner). Scale bar, 10µm.*

a more comprehensive view of the panglial network and its heterogeneity and function in the brain. (Funded by DFG).

The functional expression of transmitter receptors in microglia

Maria Pannell, Stefan Wendt, Laura Korvers, Marina Matyash, Vitali Matyash, Susanne A. Wolf

We have addressed the question whether microglia would also express receptors to sense neuronal activity. We have previously identified receptors for the neurotransmitters/neurohormones GABA, adrenaline, dopamine, bradykinin, serotonin, endothelin-1, substance P and histamine. We found that activation of these receptors can modulate microglial functions such as migration or cytokine release. Using freshly isolated microglia from healthy adult mice, we found that only a small fraction (1 - 20 %) responded to the application of endothelin, histamine, substance P, serotonin, galanin, somatostatin, angiotensin II, vasopressin, neurotensin, dopamine or nicotine. In cultured microglia from neonatal and adult mice, a similarly small population of cells responded to these neurotransmitters/-hormones. To induce a pro-inflammatory phenotype, we applied LPS or IFN-γ to the cultures for 24 h. Several of the responding populations increased, but there was no uniform pattern when comparing adult with neonatal microglia or LPS with IFN-γ treatment. IL-4 as an anti-inflammatory substance increased the histamine, substance P and somatostatin sensitive populations only in microglia from adult, but not in neonatal cells. We also found that the expression of different receptors was not strongly correlated indicating that there are many different populations of microglia with a distinct set of receptors. A subpopulation (11%) of freshly isolated adult microglia respond to the muscarinic acetylcholine receptor agonist carbachol with a Ca^{2+} increase, indicating the expression of functional receptors. The carbachol-

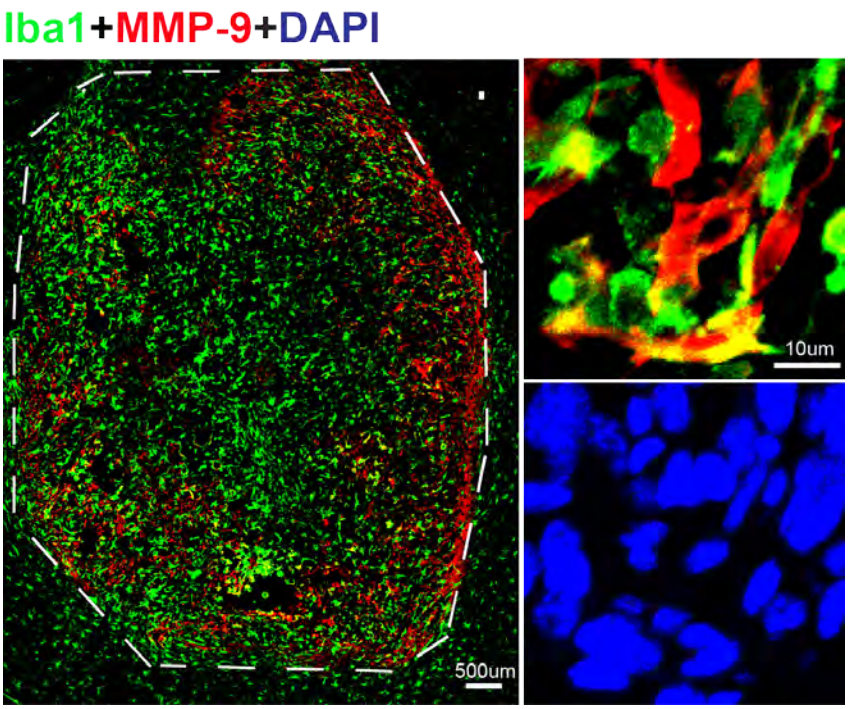


Figure 2:
Mouse brains injected with GL261 glioma cells were stained for the microglial marker Iba-1(green) and for metalloprotease-9 (red). In the tumor free area the level of metalloprotease-9 was low (left, outside of the dashed line) while within the tumor metalloprotease-9 is expressed mainly in Iba-1 positive cells (right).

sensitive population increased in microglia/brain macrophages isolated from the tissue of mouse models for stroke (60%) and Alzheimer's disease (25%), but not for glioma and multiple sclerosis. Microglia cultured from adult and neonatal brain contained a small carbachol-sensitive subpopulation (8% and 9%), which was increased by treatment with interferon-γ to around 60% after 12 hours. This increase was sensitive to blockers of protein synthesis and correlated with an upregulation of the M3 receptor subtype. Carbachol was a chemoattractant for microglia and decreased their phagocytic activity. Thus microglia are a heterogeneous population with respect to chemosensitivity, and the carbachol-sensitive population expands in defined diseases. (Funded by DFG).

The response of microglial cells to brain injury

Petya Georgieva, Philipp Jordan, Anaïs Djodari-Irani, Susanne A. Wolf

Microglial cells are the pathologic sensors and represent the immune cells of the central nervous system. During any kind of disease or any pathological event such as after trauma, stroke or in multiple sclerosis, the resting microglial cell transforms into an activated form characterized by an ameboid morphology. Activated microglia can proliferate, migrate to the site of injury, phagocytose, and release a variety of factors like cytokines, chemokines, nitric oxide and growth factors. We are studying microglial properties in different mouse models of disease including Alzheimer's disease (with Frank Heppner, Neuropathology, Charité) and stroke (in collaboration with Matthias Endres, Neurology, Charité). In collaboration with Christine Winter (TU Dresden and Charité) we study the impact of deep brain stimulation on microglial properties. We are currently investigating whether microglia are activated in the projection areas of the stimulated target. (Funded by DFG)

Microglia as therapeutic target in psychiatric disorders

Daniele Mattei, Anaïs Djodari-Irani, Marina Matyash, Susanne A. Wolf

Many studies discuss an immunological component in the onset and progression of psychiatric disorders. While the peripheral immune system has been intensively investigated, little is known about the role of microglia, the intrinsic immune competent cells of the brain. We thus aimed to study the microglia phenotype derived from rodent models of schizophrenia and depression including epigenetic factors. We used a maternal immune stimulation rodent model of schizophrenia in which polyinosinic-polycytidilic acid (Poly I:C) was injected into pregnant rats to mimic an anti-viral immune response. We identified microglia IL-1β and TNF-α increase constituting the factors correlating best with decreases in net-neurogenesis and impairment in pre-

pulse inhibition of a startle response in the Poly I:C model. Treatment with the antibiotic minocycline (3mg/kg/day) normalized microglial cytokine production in the hippocampus and rescued neurogenesis and behavior. We could show that enhanced microglial TNF-α and IL-1β production in the hippocampus was accompanied by a decrease in the pro-proliferative TNFR2 receptor expression on neuronal progenitor cells, which could be attenuated by minocycline. These findings strongly support the idea to use anti-inflammatory drugs to target microglia activation as an adjunctive therapy in schizophrenic patients.

Do microglial cells influence glioma cells?

Feng Hu, Frank Szulzewsky, Felipe Sassi, Susanne A. Wolf (In collaboration with Michael Synowitz, Neurosurgery, Charité, and Dolores Hambardzumyan, Cleveland Clinic, Ohio, USA)

Gliomas comprise the majority of cerebral tumors and patients have a poor prognosis since there is essentially no concept for successful treatment. Gliomas include astrocytomas, oligodendrogliomas, and the most malignant (and untreatable) brain tumor, the glioblastoma multiforme. A large fraction of the cells within a glioma are brain macrophages/microglial cells which can amount up to 30%. We have found that microglial cells strongly promote glioma growth and invasion. There is an interesting interplay between microglial and glioma cells. Glioma cells release the metalloprotease MMP2 which is important for degradation of extracellular matrix and promotes invasion. This metalloprotease is, however, released in an inactive, larger form and it needs to be cleaved to acquire its activity. This cleavage is accomplished by the ectoenzyme MT1-MMP. A factor released by the glioma cells activates TLR2 toll-like receptors in the surrounding microglial cells and triggers the expression of MT1-MMP. Thus, glioma cells exploit microglial cells to promote their invasion. Thus interfering with TLR receptors or their intracellular pathways might reduce the rapid expansion of glioma cells and microglia have become a new target for glioma research

We have established a procedure to purify and culture human and mouse glioma-associated and non-associated microglial cells. Corresponding control (non-glioma–associated microglial cells) human cells are obtained from patients with epileptic seizures that underwent temporal lobe surgery. In the mouse model we also distinguish between blood-derived monocytes and microglia invaded from the brain parenchyma. Genetic profiling of the different microglial populations will indicate new pathways of microglia glioma interaction. We also determine how bulk glioma cells and glioma stem cells differentially affect the microglial phenotype. We will try to identify potential therapeutic targets that interfere with pathways of microglia glioma interactions with the ultimate goal to convert microglia from a pro- to an anti-tumorigenic phenotype. (Funded by DFG and NIH)

Selected Publications

Krabbe G., Matyash V., Pannasch U., Mamer L., Boddeke HW., Kettenmann H. (2012) Activation of serotonin receptors promotes microglial injury-induced motility but attenuates phagocytic activity. Brain Behav Immun. 26:419-428.

Stock K*, Kumar J*, Synowitz* M, Petrosino S, Imperatore R, Smith ESJ, Wend P, Purfürst B, Nuber UA, Gurok U, Matyash V, Wälzlein JH, Chirasani SR, Dittmar G, Cravatt BF, Momma S, Lewin GR, Ligresti A, De Petrocellis L, Cristino L, Di Marzo V, Kettenmann H*, Glass R* (2012) Neural precursor cells induce cell-death of high-grade astrocytomas via stimulation of TRPV1. Nat. Med., 18:1232 - 1239. * These authors contributed equally

Tress O., Maglione M., May D., Pivneva T., Richter N., Seyfarth .J, Binder S., Zlomuzica A., Seifert G., Theis M., Dere E., Kettenmann H*, and Willecke K* (2012) Panglial gap junctional communication is essential for maintenance of myelin in the central nervous system. J. Neurosci., 32:7499-7518, * These authors contributed equally

Krabbe*, G., Halle*, A., Matyash, V., Rinnenthal, J. L., Eom, G. D., Bernhardt, U., Miller, K. R., Prokop, S. Kettenmann#, H., Heppner# F. L. (2013) Functional impairment of microglia coincides with beta-amyloid deposition in mice with Alzheimer-like pathology. Plos One, 8:e60921. doi: 10.1371in press. #,* equal contribution

Kettenmann H., Kirchhoff F. and Verkhratsky A. (2013) Microglia: New roles for the synaptic stripper. Neuron 77: 10 – 18.

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Photo: Peter Sonnabend/NGFN

Erich Wanker

Proteomics and Molecular Mechanisms of Neurodegenerative Diseases

1 The UBX protein ASPL rapidly converts functional p97 hexamers into non-functional heterotetramers

P97/VCP is a multifunctional ATPase that interacts with various adaptor proteins and influences their cellular function in a spatio-temporal manner. However, the number, size and biological properties of p97/VCP containing protein complexes in mammalian cells are currently unknown. Recently, we have identified ten novel p97/VCP associated proteins by automated Y2H interaction screening; one of these - the UBX containing protein ASPL - was characterized in detail. We found that ASPL directly associates with VCP hexamers and rapidly converts these structures into highly stable VCP:ASPL heterotetramers. The crystal structure of p97/VCP bound to a C-terminal fragment of ASPL revealed that a unique helical lariat structure extending the UBX domain is crucial for the disruption of inter-protomer interactions and the disassembly of p97/VCP hexamers. Site-directed mutagenesis of residues facilitating the VCP:ASPL interaction abrogated the disassociation of hexamers. Overexpression of wild-type ASPL in mammalian cells induced the activation of effector caspases, which was not observed with a mutant ASPL variant that does not disassemble p97/VCP hexamers *in vitro.*

Thus, our investigations provide biochemical and structural evidence that ASPL functions as a highly specific hexamer segregase, controlling the cellular activity of p97/VCP.

2 Identification of small molecule modulators of light chain aggregates in AL amyloidosis

In the context of protein misfolding disorders, the study of a disease called amyloid light chain amyloidosis (AL amyloidosis), has only recently entered our lab. In AL amyloidosis the overproduction of immunoglobulin light chains in B cells leads to the formation of amyloid deposits, most commonly in the heart and kidney, causing dysfunction and failure. As the initial symptoms are subtle, early disease diagnosis is difficult. Consequently, patients suffering from AL amyloidosis have a short life expectancy after diagnosis. To date, the most common therapy is to destroy the overproliferating monoclonal plasma cells by chemotherapy. However, there are currently no therapies that have a curative effect. Therefore, new therapeutic strategies for this severe disease are of high interest. The focus of our project is the identification of small molecule compounds, which can either actively remove the toxic amyloid deposits or promote their degradation.

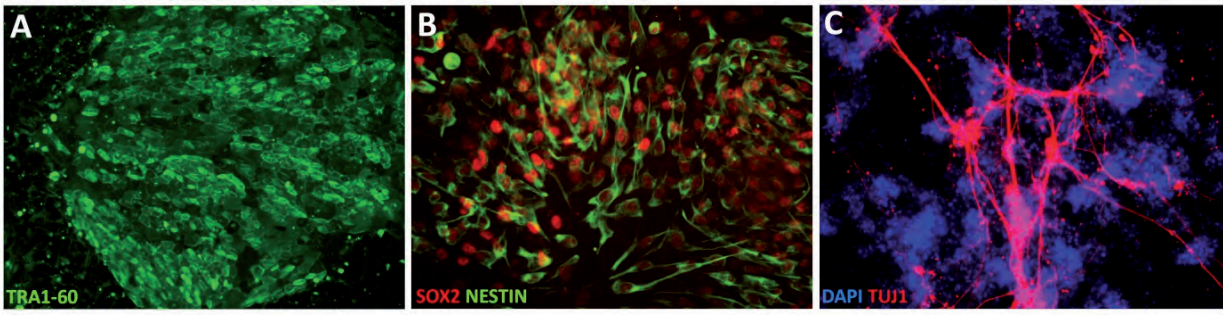


Figure 1. Induced pluripotent stem (iPS) cells are differentiated in neural precursor cells (NPCs). *(A) iPS cells derived from patients affected by Leigh syndrome (LS) and Huntington's disease (HD) express pluripotency associated markers including TRA1-60. (B) Multipotent NPCs, positive for NPC markers such as SOX2 and NESTIN, are obtained from LS-iPS cells and HD-iPS cells. (C) Patient and control NPCs are coaxed into post-mitotic neurons expressing TUJ1 and other specific markers of basal ganglia.*

3 Induced pluripotent stem (iPS) cell-based modelling of neurodegenerative disorders

The technology of induced pluripotent stem (iPS) cells may become a novel paradigm in drug discovery for brain diseases by allowing live patient neurons to be employed in phenotype-based drug screening. We are exploring this approach by generating iPS cells from two genetic disorders affecting the basal ganglia: Leigh syndrome and Huntington's disease. iPS cells are established from patients using a footprint-free episomal plasmid-based reprogramming protocol. Patient iPS cells are then differentiated into neural precursor cells (NPCs), which are highly proliferative and retain their multipotent nature for several passages. Since current methods for NPC derivation are complex and time-consuming, we have developed a small molecule-based protocol that converts iPS cells into NPCs in a rapid and adherent fashion (Bukowiecki et al, in preparation). Leigh syndrome-NPCs and Huntington's disease-NPCs are currently being differentiated into basal ganglia neurons, whose identity and functional properties are under evaluation (Fig. 1). Finally, by combining standard functional and biochemical assays with global -omics-driven analyses on patient-derived neurons, we wish to unravel the Leigh syndrome and Huntington's disease mechanisms and potentially identify novel therapeutic strategies for these debilitating disorders.

4 The protein interactome of the chemical synapse

Central to all neuronal functions are the trillions of chemical synapses within the human brain that transmit chemical signals. Signal transmission is performed and regulated by complex protein networks in the pre- and post- synaptic termini. In the past, efforts in synaptic proteomics have been focused on identifying the constituents of the synaptic sub-compartments. Over 2000 gene products have been identified, but their binary interactions have not previously been studied on a large scale. To gain more insight into multi-protein complexes formed at the synapse we have adopted a high-throughput screening strategy; approximately 1000 proteins are being tested for pair wise interaction using the yeast-2-hybrid technique.

Following Y2H screening, selection of interacting protein pairs for validation will be based upon a scoring system which takes into account six factors: 1) known interactions between orthologues, 2) the presence of domains known to interact in each of the

proteins, 3) co-expression, 4) co-localisation, 5) participation in the same biological process and 6) distance in an interaction network made up of all known interactions. High confidence interactions will be validated using the LUMIER co-immunoprecipitation method.

The protein interaction network generated by this study will be a useful resource for studies on synapse dysfunction, which is central to the etiology and progression of several neurological disorders including neurodegeneration, autism and schizophrenia.

5 Protein-protein interaction studies using a split-ubiquitin yeast-3-hybrid assay

This project encompasses both method development and biochemical investigation. We are validating a new strategy for detection of protein-protein interactions: the split-ubiquitin yeast-3-hybrid assay. This assay involves co-expression of a constitutively active kinase with two potentially interacting proteins within a yeast cell. The effect of the kinase on the protein interaction event is monitored through activation of the reporter genes HIS3 and β-GAL.

Our study focuses on phosphorylation of Munc-18 (STXBP1), a synaptic protein that plays a critical role in synaptic vesicle docking at the pre-synaptic membrane prior to neurotransmitter release. It has been shown that phosphorylation of Munc-18 by protein kinase C alpha (PKCα) causes it to release the SNARE protein, syntaxin-1, allowing the SNARE protein complex to form. The SNARE complex then mediates both vesicle docking and neurotransmitter release. To further understand the sequence of events that regulate this process we aim to identify proteins that interact with Munc-18 in a manner regulated by PKCα.

6 Interaction network filtering identifies CRMP-1 as a suppressor of mutant huntingtin misfolding and neurotoxicity

Assemblies of huntingtin fragments with expanded polyglutamine tracts are a pathological hallmark of Huntington's disease. The molecular mechanisms of how these structures cause neuronal dysfunction and toxicity are poorly understood. We utilized gene expression information from specific brain regions in health and disease for systematic filtering of a protein-protein interaction data set. Our step-by-step computational strategy facilitated the generation of a small, focused interaction network, which directly or indirectly connects 13 potentially dysregulated, brain-region-specific proteins with the disease protein huntingtin. This network enabled the discovery of the heat shock inducible neuron-specific protein CRMP-1, which targets aggregation-prone, N-terminal huntingtin fragments and suppresses their spontaneous self-assembly into proteotoxic structures in various disease models. We propose that our computational network filtering procedure is a simple but highly valuable strategy for predicting potentially disease-relevant proteins that influence misfolding and neurotoxicity of polyglutamine disease proteins.

7 Interactome mapping identifies the oncoprotein BMI1 as a stimulator of autophagosome formation

Autophagy is an evolutionarily conserved catabolic process involved in the degradation and recycling of proteins and damaged organelles by the lysosome. Currently, the molecular mechanisms that control autophagy induction are poorly understood. We generated a binary yeast two hybrid (Y2H) interaction network, connecting 1177 human proteins via 2407 high-quality interactions to the autophagy machinery.

This network facilitated the discovery of the nuclear oncoprotein BMI1 that interacts with ATG12, a critical mediator of autophagosome formation. Strikingly, BMI1, which shuttles from the nucleus to the cytoplasm under stress conditions and targets ATG12 through its RING domain, is a potent stimulator of autophagosome biogenesis in various cell model systems. Cytoplasic BMI1 protein levels are increased in mammalian cells that harbor misfolded, proteotoxic protein aggregates of huntingtin, a protein central to pathogenesis of the neurodegenerative disorder Huntington's disease (HD). Expression and subcellular localization of BMI1 are altered in brains of HD patients. We propose a model in which nuclear proteins such as BMI1 directly regulate autophagy induction in mammalian cells and stimulate the degradation of misfolded, proteotoxic proteins. These results could be highly relevant to therapy development, both for cancer and neurodegenerative diseases.

Selected Publications

Petrakis, S., Rasko, T., Russ, J., Friedrich, R.P., Stroedicke, M., Riechers, S.P., Muehlenberg, K., Moeller, A., Reinhardt, A., Vinayagam, A., Schaefer, M.H., Boutros, M., Tricoire, H., Andrade-Navarro, M.A., Wanker, E.E. (2012). Identification of human proteins that modify misfolding and proteotoxicity of pathogenic ataxin-1. PLoS Genetics 8 (8): e1002897.

Vinayagam, A., Stelzl, U., Foulle, R., Plassmann, S., Zenkner, M., Timm, J., Assmus, H.E., Andrade-Navarro, M.A., Wanker, E.E. (2011). A directed protein interaction network for investigating intracellular signal transduction. Sci Signal. 4 (189): rs8.

Bieschke, J., Herbst, M., Wiglenda, T., Friedrich, R.P., Boeddrich, A., Schiele, F., Kleckers, D., Lopez del Amo, J.M., Grüning, B.A., Wang, Q., Schmidt, M.R., Lurz, R., Anwyl, R., Schnoegl, S., Fändrich, M., Frank, R.F., Reif, B., Günther, S., Walsh, D.M., Wanker, E.E. (2011). Small-molecule conversion of toxic oligomers to nontoxic β-sheet-rich amyloid fibrils. Nat Chem Biol. 8(1):93-101.

Bieschke, J., Russ, J., Friedrich, R.P., Ehrnhoefer, D.E., Wobst, H., Neugebauer, K., Wanker, E.E. (2010). EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity. Proc Natl Acad Sci U S A. 107(17):7710-5.

Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F.H., Goehler, H., Stroedicke, M., Zenkner, M., Schoenherr, A., Koeppen, S., Timm, J., Mintzlaff, S., Abraham, C., Bock, N., Kietzmann, S., Goedde, A., Toksoez, E., Droege, A., Krobitsch, S., Korn, B., Birchmeier, W., Lehrach, H., Wanker, E.E. (2005). A human protein-protein interaction network: a resource for annotating the proteome. Cell 122 (6): 957-968

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Photo: David Ausserhofer/MDC

Annette Hammes

Molecular Pathways in Cortical Development

Receptor-mediated endocytosis provides the main mechanism whereby cells take up metabolites and signaling molecules from the extracellular space. One class of endocytic receptors, termed low-density lipoprotein receptor-related proteins (LRPs), is particularly relevant for embryonic brain development. We investigate the role of LRPs and their interaction with morphogens and signaling molecules to regulate developmental events during early cortical development. In particular we are interested in how endocytic receptor dysfunction leads to developmental disturbances of the CNS.

LRP2 is an auxiliary SHH receptor required for cortical development

Annabel Christ, Anna Christa in collaboration with Prof. Thomas Willnow

LRP2, the largest member of the LRP family, is a multifunctional endocytic receptor expressed on the apical surface of the entire neuroepithelium during neurulation (Figure 1). Loss of LRP2 activity in the developing CNS in gene targeted mice causes holoprosencephaly (HPE), a failure of the forebrain hemispheres to separate along the midline. Patients with autosomal recessive *LRP2* gene defects suffer from Donnai-Barrow syndrome, a disorder associated with forebrain anomalies. In collaboration with the group of Prof. Thomas Willnow we previously clarified the molecular mechanism underlying the HPE phenotype by identifying LRP2 as a novel component of the sonic hedgehog (SHH) signaling machinery in the ventral forebrain neuroepithelium. LRP2 acts as a SHH binding protein and co-receptor to patched1 that sequesters SHH at the apical surface of the neuroepithelium and controls internalization and cellular trafficking of SHH to recycling endosomes (Christ et al., 2012). LRP2 deficiency in mice leads to failure of the ventral neuroepithelium to respond to the morphogen SHH and consequently to improper specification of forebrain midline structures. Our data substantiate the emerging concept that auxiliary receptors are critical modulators of morphogen delivery and signal reception in target tissues, and identified an important role for LRP2 in SHH action in the ventral forebrain (Figure 2).

Endocytic receptor mediated control of folate uptake during neurulation

Nora Mecklenburg, Esther Kur

Having established a role for LRP2 in SHH signaling in the ventral midline of the rostral neural tube, the function of the receptor in lateral and dorsal neural tube domains, however, remained unclear. Cranial neural tube defects (NTDs) in LRP2 deficient mice, unrelated to the etiology of HPE, hinted at an additional role for LRP2 in receptor mediated uptake of a specific ligand in the developing neural tube. Thirty-eight percent of LRP2 deficient embryos show impaired closure of the rostral neural tube. Similar neural tube defects have previously been attributed to impaired folate (vitamin B_9) metabolism in mice and humans. Neural tube defects are a group of congenital malformations that occur when the neural tube fails to close during embryonic development. In human pregnancies, NTDs are the second most frequent malformations after congenital heart defects.

We therefore asked whether LRP2 might be required for delivery of folate to neuroepithelial cells during neurulation. Uptake assays in whole embryo cultures showed that LRP2 deficient neuroepithelial cells are unable to me-

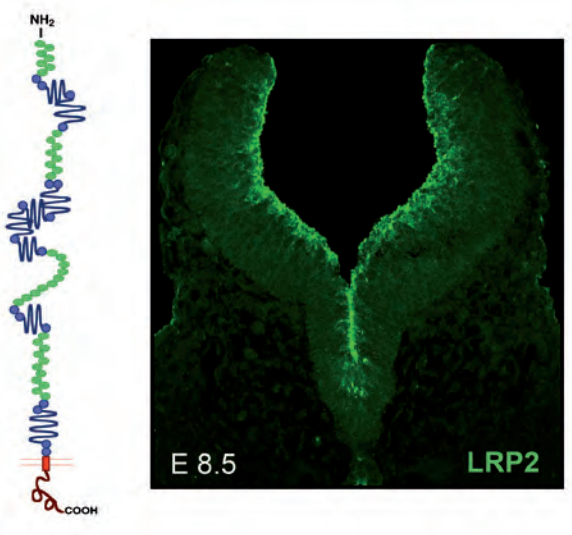


Figure 1: Expression of LRP2 in the developing mouse forebrain. *The scheme depicts the structure of LRP2, a single-spanning transmembrane endocytic receptor of the LDL receptor gene family. Immunohistological detection of LRP2 (green) on coronal sections of the rostral neural tube of the mouse embryo shows that the receptor is expressed uniformly on the apical neuroepithelial surface.*

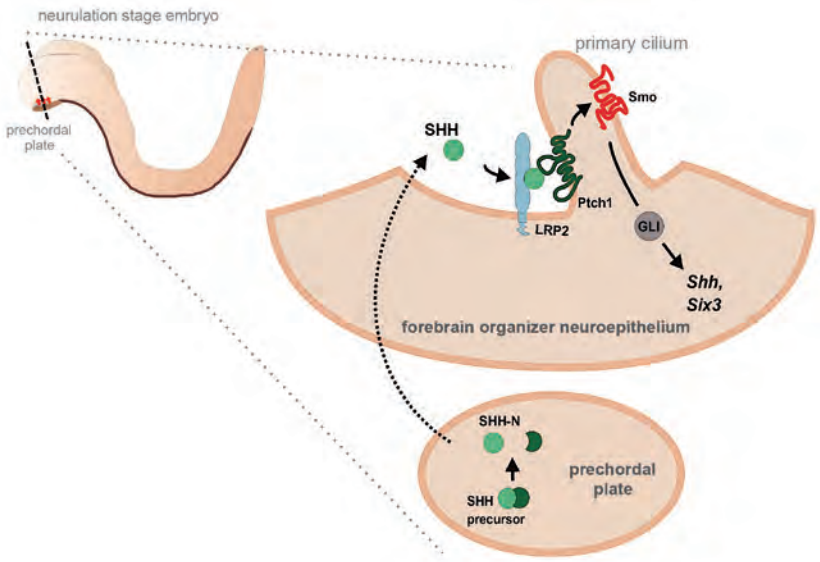


Figure 2: Model of LRP2 function in SHH signaling. *SHH produced in the prechordal plate moves to the neuroepithelium to be sequestered apically by a co-receptor complex of LRP2 and patched 1 (Ptch1). SHH binding induces uptake of receptor-ligand complexes and triggers Ptch1-dependent activation of smoothened (Smo) resulting in target gene induction through GLI.*

diate uptake of folate bound to folate receptor 1 (FOLR1).

So far a putative co-receptor for FOLR1 had not yet been identified. Our data suggest that endocytosis of soluble FOLR1 and the membrane anchored GPI-FOLR1, requires interaction with the endocytic receptor LRP2. This "dual receptor complex" hypothesis for the uptake of folate is analogous to the uptake of renal and intestinal vitamin B_{12} bound to intrinsic factor (IF) via cubilin, a peripherally attached glycoprotein. Internalization of the cubilin-ligand complex strongly relies on the interaction with LRP2.

Our results demonstrate that LRP2 plays an essential role for the uptake of folate in the developing neural tube, a crucial step for proper neural tube closure. LRP2 deficiency leads to impaired cellular uptake of folate during neurulation and reduced tissue folate concentrations, which consequently contributes to NTDs in *Lrp2*$^{-/-}$ embryos.

Perspectives

Future work aims at substantiating the relevance of LRP2 as part of the SHH receptorsome in developmental pathways of medical relevance including forebrain development and HPE, retina formation and retinopathies, as well as adult neurogenesis.

Further we expect to elucidate the complex interaction of LRP2 with other receptors, like the folate receptor, to convert extracellular stimuli into intracellular signals. Shedding light on the mechanisms how LRP2 and other members of the LDL receptor family can function in a dual receptor mode to confer ligand specificity in a context dependent manner in the developing neural tube will contribute to the emerging concept that LRPs are critical modulators of signal reception.

Selected Publications

Kur, E., Mecklenburg, N., Cabrera, R.M., Willnow T.E., and Annette Hammes. (2014) LRP2 mediates folate uptake in the developing neural tube J Cell Science March 17

Christ, A., Christa, A., Kur, E., Lioubinski, O., Bachmann, S., Willnow, T.E., and Hammes, A. (2012). LRP2 is an auxiliary SHH receptor required to condition the forebrain ventral midline for inductive signals. Dev Cell 22: 268-278

Willnow, T.E., Christ, A., and A. Hammes (2012). Endocytic receptors control morphogen signalling. Development 139(23): 4311-4319.

Gajera, C.R., Emich, H., Lioubinski, O., Christ, A., Beckervordersandforth-Bonk, R., Yoshikawa, K., Bachmann, S., Christensen, E.I., Götz, M., Kempermann, G., Peterson, A.S., Willnow, T.E. and A. Hammes. (2010). Ependymal cells regulate BMP signaling in the adult neurogenic niche through LRP2. J. Cell Science 123: 1922-30.

Hammes, A., Andreassen, T. K., Spoelgen, R., Raila, J., Huebner, N., Schulz, H., Metzger, J., Schweigert, F. J., Luppa, P. B., Nykjaerr, A. and T. E. Willnow. (2005) Impaired development of the reproductive organs in mice lacking megalin, an endocytic receptor for steroid hormones. Cell 122: 751-62.

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Photo: David Ausserhofer/MDC

Kathryn Poole

Cellular Sensing of the Physical Environment

The process by which cells can convert mechanical and physical stimuli into a biochemical response is known as mechanotranduction, a broad term that covers a number of processes involved in cellular sensing of the physical environment. These include the fast channel-mediated transduction of mechanical stimuli into electrical responses (mechanoelectrical transduction) to slower transduction events mediated by intracellular force-sensing proteins associated with the cytoskeleton or integrins at the cell surface. Mechanotransductive mechanisms are not only fundamental to our senses of touch and hearing but also to cellular processes such as differentiation, migration and tissue formation and the disruption of mechanotransduction pathways can have a negative impact on health, implicated in osteoarthritis, cancer development and metastasis and mechanical dysfunction of the heart, to name but a few.

We are interested in how individual cells detect and respond to applied forces and to the physical features of their immediate environment, namely the extracellular matrix (ECM). In the last years we have been using a number of biophysical techniques to be able to better characterize the physical features of the ECM that cells can detect and how these signals are transduced across the membrane. Initially this work was carried out in mammalian somatosensory neurons, i.e. those cells that mediate our sense of touch. More recently we have been extending this work to investigate the role of mechanically-gated channels in cell migration and chondrocyte mechanotransduction.

Mechanotransduction in sensory neurons is influenced by the laminin network to which the cells are bound. Laminins are major components of basement membranes, an ECM-based compartment with which neurites from DRG cells are associated in vivo. Using micro-contact printing to pattern surfaces with specific laminin mixtures we were able to polarize cells in terms of both neurite outgrowth and mechanotransdution. We found that by spiking an EHS-laminin substrate with laminin-332 the EHS-laminin became inhibitory for both growth-cone bifurcation and mechanotransduction (Fig 1). Not only did these results elucidate two novel functions of laminin-332 they also demonstrate that there is a contact-dependant effect on mechanotransduction that is a local, not a global phenomenon. This suggests that mechanically-gated ion channels are primarily activated at the cell-substrate interface. An atomic force microscopy analysis of the effect of adding laminin-332 to EHS-laminin demonstrated that increasing amounts of laminin-332 led to a decrease in stability of the EHS-laminin network by modulating its topography and rigidity[1]. This suggests that the physical features of the substrate are critical in supporting robust mechanotransduction. We are currently testing this hypothesis using a panel of laminin substrates that vary in either their composition or their structure.

Our investigation of the influence of local cell-matrix interactions on mechanotransduction highlighted a need for a new approach to quantify mechanoelectrical transduction where the physical stimuli are applied at the relevant cellular compartment. In order to achieve this we have developed a highly sen-

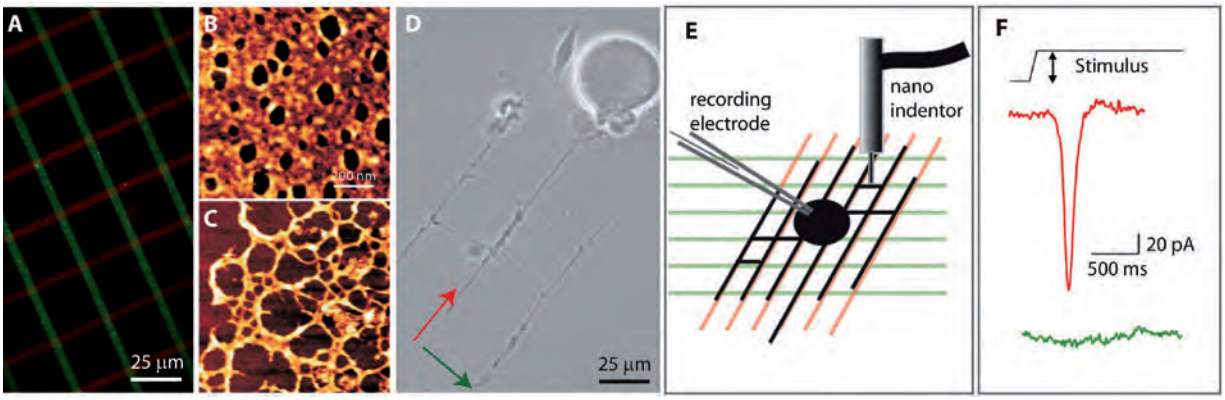


Figure 1. Laminin-directed polarization of DRG sensory neurons. *Distinct mixes of laminin subtypes can be printed on a surface using microcontact printing (A- red, EHS laminin; green, EHS laminin: laminin 332, 15:1) AFM can be used to image structural properties of these substrates (B, EHS laminin; C, EHS laminin: laminin 332, 15:1). Neurite outgrowth is polarized on these substrates (D). Neurite segments from a single cell, over each distinct substrate, can be probed (E), resulting in either presence (F, red trace, EHS laminin) or absence of a response (F, green trace EHS laminin: laminin 332, 15:1)*

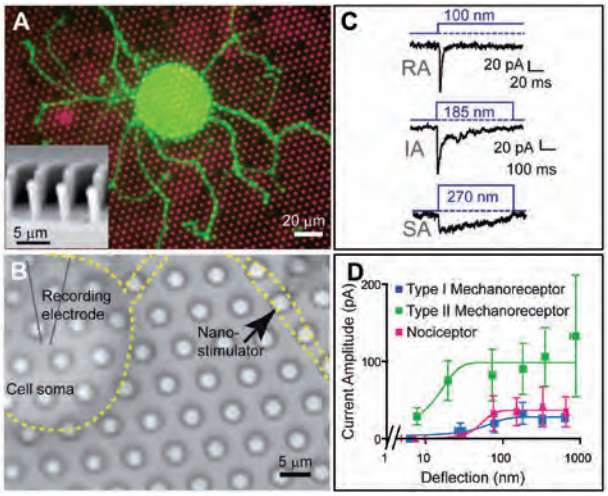


Figure 2: Quantitative measurement of mechanoelectrical transduction. *(A) A pillar array is cast from the elastomer PDMS (insert), coated with ECM molecules and cells, shown here are sensory neurons, can be cultured on top of the pillar array. (B) Individual pili underneath the cell can then be deflected using a nano-stimulator and mechanically-gated channel activity is monitored using whole-cell patch-clamp. (C) This method is particularly sensitive and quantitative, as the precise movement of the stimulating pili can be calculated and correlated to the resulting currents. (D) Stimulus-response curves of mechanotransduction in subsets of sensory neurons. Note that TypeII mechanoreceptors are extremely sensitive to mechanical stimuli.*

sitive method where precise, molecular-scale stimuli are applied to defined regions of the cell-matrix interface[2]. Briefly, a pillar array is cast in the elastomer PDMS and the tops of the pili are printed with ECM molecules upon which the cells of interest (in this case somatosensory neurons) are cultured (Fig 2). Stimulus-response curves are generated by deflecting an individual pilus underneath a neurite or cell segment and measuring the cellular response using whole-cell patch-clamp. Using this system, we have been able to precisely measure the amplitude of mechano-electrical transduction currents in response to defined stimuli. We have found that some cells can transduce substrate deflections of only 13 nm, or half the width of a microtubule. As such, these cells can detect molecular-scale stimuli. We found that this sensitivity was dependent on the presence of a membrane scaffolding protein (STOML3), which functions to tune the sensitivity of ion channels that are directly gated by mechanical stimuli[2]. These data indicate that mechanically-gated channel thresholds can be modulated by a scaffolding protein, suggesting a mechanism by which different cell types can tune their sensitivity to various mechanical stimuli. We are currently using this technique to characterize the role of mechanically-gated ion channels in chondrocyte mechanotransduction and in proliferative cell migration.

[1] Chiang, L-Y*., Poole, K*., et al (2011) Nat. Neurosci. 14, 993-1000.
[2] Poole, K. et al (2014) Nat. Comm. In press.

Selected Publications

K. Poole§, R. Herget, L. Lapatsina, HD. Ngo, G.R. Lewin§. (2014) Nanoscale tuning of mechanosensitive ion channels by stomatin-domain proteins. Nat Comm (2014) 5: doi:10.1038/ncomms4520

J. Brand, E. St. J. Smith, D. Schwefel, L. Lapatsina, K. Poole, A. Kozlenkov, J. Behlke, G. R. Lewin and O. Daumke (2012) A stomatin dimer modulates the activity of acid-sensing ion channels. EMBO J. **31:** 3635 - 3646

S.G. Lechner* ,S. Markworth*, K. Poole, E. St.J. Smith, L. Lapatsina, S. Frahm, M. Suzuki, I. Ibañez-Tallon, F.C. Luft, J. Jordan and G.R. Lewin (2011) The molecular and cellular identity of peripheral osmoreceptors. Neuron **69:** 332-344

L.Y. Chiang*, K. Poole*, B.E. Oliveira, N. Duarte, Y.A. Bernal-Sierra, L. Bruckner-Tuderman, M. Koch, J. Hu and G. R. Lewin (2011) Laminin-332 coordinates mechanotransduction and growth cone bifurcation in sensory neurons. Nat. Neurosci. **14:** 993–1000

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Prevalent and devastating diseases such as cancer, cardiovascular disease, metabolic diseases and neurodegenerative disorders are usually multifactorial diseases involving many genes. To extend our understanding of the interplay of functions involving different genes in health and disease, an important goal of systems biology is to analyze gene regulatory networks. To this end we collect genome-wide quantitative data to integrate them into predictive models. This challenge is at the heart of Medical Systems Biology and is by nature highly interdisciplinary, combining molecular and cellular biology, biochemistry, mathematics, physics and engineering. The past ten years have particularly highlighted that cells employ multiple sophisticated levels of gene regulation, which include organization of chromosomes in the nucleus, epigenetic gene regulation, transcription, post-transcriptional gene regulation and post-translational modifications. Importantly, all of these levels interact and regulate each other and are involved in disease development. At the MDC, the grand challenge and Scientific Mission of the "Berlin Institute for Medical Systems Biology" (BIMSB) is to integrate these levels of gene regulatory control into comprehensive and predictive models of how gene regulatory changes are linked to phenotypes.

Scientific Technology Platforms

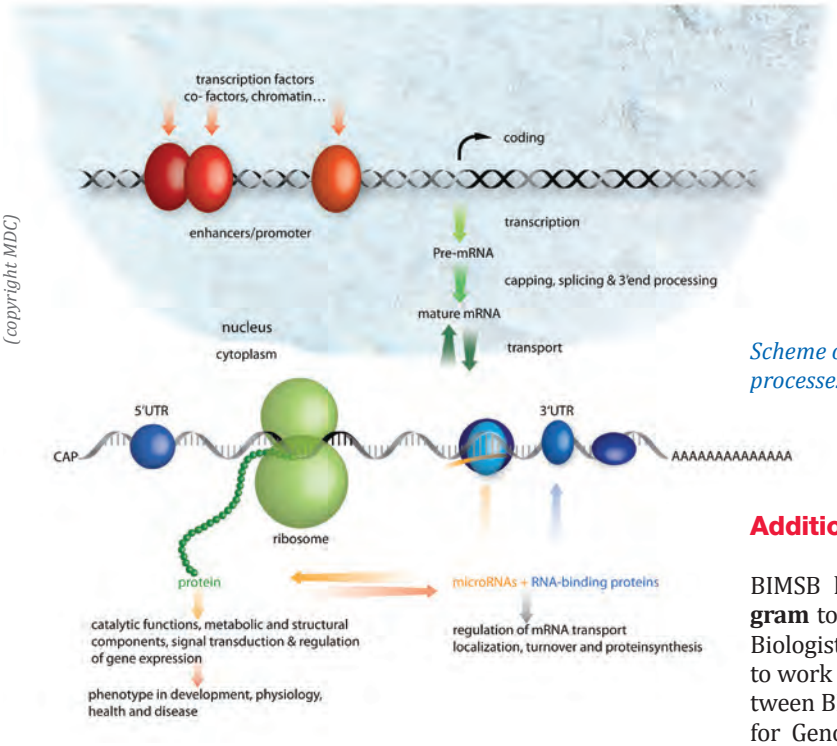
High throughput technologies are key for many research aspects at BIMSB, we have therefore established essential Scientific Technology Platforms for 'omics'-technologies. The newly recruited PIs have installed and integrated these technologies in scientific projects, often in collaboration with other labs at the BIMSB/MDC. **Wei Chen** directs the Genomics platform which offers diverse applications, including genome and transcriptome sequencing and assembly, and ChIP-Seq. **Stefan Kempa** develops cutting edge methods to quantify the metabolome as well as the proteome, with a special focus on aspects of the metabolism of tumors and stem cells. The Bioinformatics Platform was headed by **Christoph Dieterich** until August 2013 and will be led by **Altuna Akalin** starting in spring 2014. Additional Technology Platforms will be established in the future.

Scientific group leaders in BIMSB

In addition to Junior group leaders **Markus Landthaler** and **Alexander Löwer,** BIMSB was fortunate to recruit several outstanding new Junior and Senior group leaders since 2012. They investigate important aspects of gene regulatory mechanisms (for example epigenetics) and model systems (e.g. Drosophila) which were not represented on campus before: **Marina Chekulaeva** 'Non coding RNA's and mechanims of cytoplasmic regulation', **Baris Tursun**, 'Gene regulation and cell fate decision in C. elegans', **Uwe Ohler** 'Computational regulatory genomics', **Ana Pombo** 'Epigenetic regulation and chromatin architecture', **Robert Zinzen** 'Systems biology of neural tissue differentiation'. **Benedikt Obermayer** 'Computation and Theory' joined BIMSB as a Max Delbrück Fellow.

Publications

BIMSB scientists have published outstanding and highly-cited papers. The scientific scope and collaborative nature within BIMSB is highlighted for example by Baltz et al. Mol Cell 2012. In this project, the Landthaler lab collaborated with the Dieterich, Selbach and Bonneau (NYU) labs, to answer a fundamental question: Can we comprehensively identify the RNA binding proteins (RBPs) which are active in a human cell? The answer is that 800 proteins can be found specifically bound to RNA in a human cell line. Numerous of these RBPs came as surprises (see also Hentze, Cell 2012). The Landthaler lab also developed a new method which quantifies which parts of RNAs in a human cell are actually bound by these RBPs. This method allows now to record transcriptome-wide "occupancy profiles" of RNA:protein interactions. Thus, the paper not only provides a much needed catalogue of the repertoire of RBPs, it also opens the door to study RNA:protein occupancy profiles in response to different conditions or stimuli. These approaches are expected to pinpoint particularly important RNA:protein interactions and to allow modeling of cross-



(copyright MDC)

Scheme of gene-regulatory processes

talk between regulatory components. The two first authors of this study are members of the BIMSB-NYU exchange program and profited from their experiences at NYU. The paper also exemplifies that often, several BIMSB/MDC scientists collaborate as lead authors on key papers. Further examples are Gruen et al. Cell Reports 2014 (Selbach, Rajewsky), Memczak et al. Nature 2013 (Landthaler, Löwer, leNoble, Rajewsky), Oenal et al. EMBO J 2013 (Chen, Kempa, Rajewsky), Doerr et al. Nature 2013 (Doerken, Kempa, Schmitt), Baltz et al. Molecular Cell 2012 (Selbach, Dieterich, Landthaler), Anders et al NAR 2012 (Rajewsky, Landthaler, Dieterich). In total BIMSB teams published 65 papers in peer-reviewed journals (2012-Feb. 2014), of which 43 papers had an impact factor (IF) higher than 5 and 13 articles an IF higher than 10 ("High-impact papers"). The 65 new papers have been cited 348 times (until Feb. 2014).

The new BIMSB building

In 2013 an open architect competition took place, based on extensive preplanning. The contruction for the winning, highly innovative architectural plan is starting in 2014 (see Fig. 2). The new building houses up to 25 research groups, approximately half experimental and half computational. On 5100 qm the building supports high-tech labs, flexible workspaces and communication areas, which are all essential for the interdisciplinary BIMSB activities. BIMSB is a key element for the Integrative Research Institute for Life Sciences IRI, part of the successful "Exzellenzinitiative" (funded by the DFG) of the Humboldt University and the Charité.

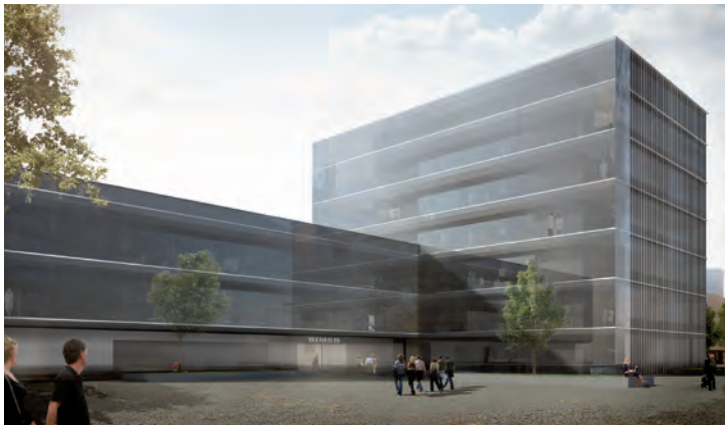
Additional Activities

BIMSB has established an **Exchange program** to train the next generation of System Biologists. The program allows PhD students to work on collaborative research project between BIMSB labs and partners at the Center for Genomics and Systems Biology at New York University. In addition to seminar series, guest seminars and lecture series, BIMSB organizes the highly successful annual **Berlin Summer Meeting**, the **Berlin Summer School**, joint retreats with the **MRC**-Clinical Sciences Centre (UK), and internal BIMSB retreats. In 2012, MDC/BIMSB PIs discussed in a two day retreat how to connect, develop, and marry traditional Genetics and Systems Biology.

Grants, Awards & Peer reviews

In addition to the start-up funding of BIMSB, the labs have successfully competed for extramural funding. Since 2012, BIMSB labs have acquired a total of 9,5 Mio € additional funds from the DFG, BMBF, EC, NIH, GIF, and others. Some of the highlights are: the **Gottfried Wilhelm Leibniz Prize** (N. Rajewsky), the **German Epigenome Consortium** 'DEEP', and BMBF funding for national projects in systems biology and bioinformatics. In two peer-reviews, scientific achievements of BIMSB as well as the overall concept were rated "outstanding".

Model of the new BIMSB building in the center of Berlin



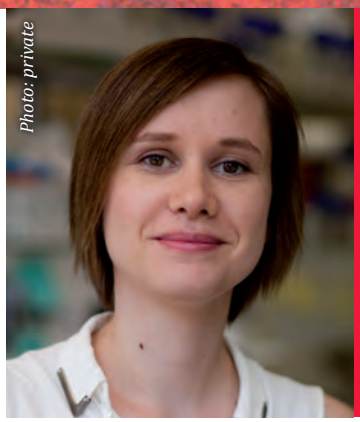


Photo: private

Marina Chekulaeva

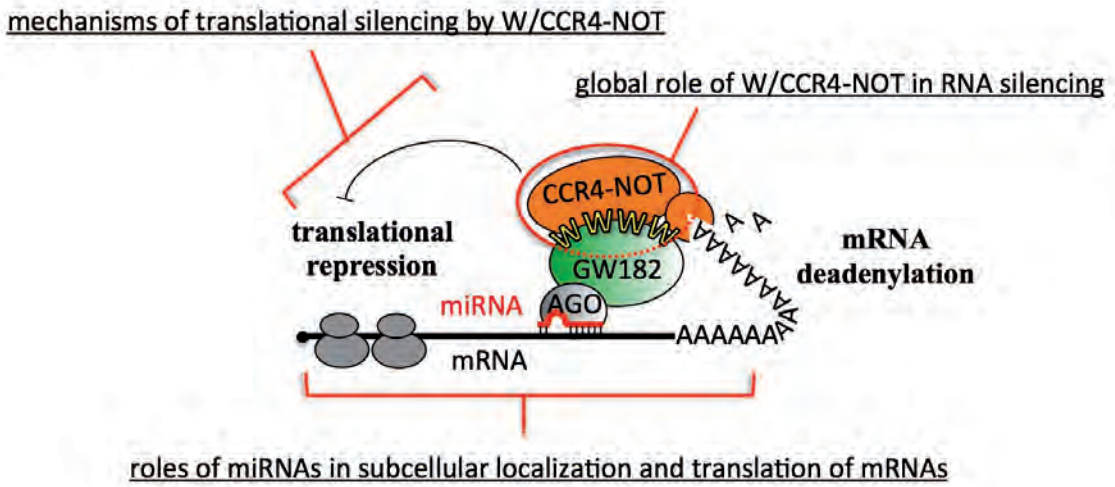
Non-coding RNAs and Mechanisms of Cytoplasmic Gene Regulation

We are focusing on understanding the molecular mechanisms that regulate mRNA translation, localization and stability and role of non-coding RNAs in this process. Up to 90% of human DNA is estimated to be transcribed into so called non-coding RNAs that are not translated into proteins. Many of them act as potent modifiers of gene expression. miRNAs are a class of such short non-coding RNAs. They regulate expression of more than a half of eukaryotic genes, thus, affecting multiple biological processes, including cell proliferation, differentiation, apoptosis and senescence. Not surprisingly, miRNAs are involved in many human pathologies, including cancer and neurological disorders and hold great potential as drug targets, disease markers, as well as therapeutic agents.

One of our interests is to understand the mechanisms of miRNA function. In animals, miRNAs regulate gene expression post-transcriptionally: they act as guides, by pairing with partially complementary sites present in their target mRNAs, and thereby recruiting a complex of proteins that inhibits mRNA function. The core components of this complex are AGO and GW182 proteins. We have shown that GW182 proteins contain a novel and conserved type of protein motifs, termed W-motifs. These motifs act in additive manner to recruit the CCR4-NOT deadenylation complex and thereby lead to translational repression, deadenylation and decay of target mRNAs. This finding reconciles literature data and provides a new foundation from which to explore miRNA function. What are the functions of the repressive W-motifs outside of the GW182 family, taking into account the pleiotropic role of the CCR4-NOT complex in RNA metabolism and its involvement in processes like cell growth, proliferation and tumorigenesis? How is miRNA function regulated in time and space? Can miRNAs regulate gene expression via alternative and previously uninvestigated mechanisms? What are the roles of extracellular miRNAs in intercellular cross-talk and propagation of diseases, such as cancer? We are using a combination of biochemical, computational approaches and imaging to address these questions.

What are the functions of the W-motifs/CCR4-NOT interaction platform in proteins outside of the miRNA repression complex?

W-motifs recruiting the CCR4-NOT complex represent a novel interaction platform employed by miRNAs to regulate gene expression. But W-motifs can be found not only in GW182, but also in many other proteins. What are their functions? Do these proteins with W-motifs function as competitors of GW182 to modulate miRNA silencing in response to various stimuli or do they regulate gene expression in a miRNA-independent way, by recruiting the CCR4-NOT complex to specific targets? The fact that CCR4-NOT can regulate multiple steps of gene expression, including transcription, chromatin



Scheme illustration the mechanisms of miRNA. *GW182 proteins are recruited to mRNA via direct interaction with the miRNA–AGO complex. Through the W-motifs, GW182 recruits the CCR4–NOT complex that represses translation an leads to mRNA deadenylation*

modification, RNA export, translation and mRNA stability, argues that proteins with W-motifs have the potential to regulate gene expression via multiple mechanisms. Using a combination of computational and biochemical approaches, we are studying the roles of the W-motif/CCR4-NOT repressive module in differential gene expression.

What are roles of miRNAs in mRNA localization and localized translation?

The role of miRNAs in translational control and mRNA decay has been well established. However, for a cell it is important not only how much of a given protein it produces, but also where in the cell this protein is produced. This is often achieved by localizing mRNAs to specific sites of the cell and repressing their translation prior to localization. These mechanisms underly establishment of body axis, cell migration, synaptic plasticity and other important processes. A number of human pathologies, including neurological disorders such as the Fragile X syndrome, are associated with a failure to localize certain mRNAs to specific subcellular compartments. To analyze the roles of miRNAs in subcellular translation and localization of mRNAs, we are using spatial transcriptomic, proteomic and bioinformatic analyses.

Selected Publications

Chekulaeva, M., Mathys, H., Zipprich, J., Attig, J., Colic, M., Parker, R., and Filipowicz, W. (2011). miRNA repression involves GW182-mediated recruitment of CCR4–NOT through conserved W-containing motifs. Nature Structural and Molecular Biology 18(11): 1218-26.

Chekulaeva, M., Parker, R., and Filipowicz, W. (2010). The GW/WG repeats of Drosophila GW182 function as effector motifs for miRNA-mediated repression. Nucleic Acids Research 38(19): 6673-83.

Chekulaeva, M. and Filipowicz, W. (2009). Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells. Current Opinion in Cell Biology 21(3): 452-60.

Chekulaeva, M., Filipowicz, W., and Parker, R. (2009). Multiple independent domains of dGW182 function in miRNA-mediated repression in Drosophila. RNA 15: 794-803.

Chekulaeva, M., Hentze, M.W., and Ephrussi, A. (2006). Bruno acts as a dual repressor of oskar mRNA translation promoting mRNA oligomerization and formation of silencing particles. Cell 124: 521-533.

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Photo: David Ausserhofer/MDC

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Video:
What can we learn from counting molecules?

Novel Sequencing Technology, Functional and Medical Genomics

"DNA makes RNA makes protein." *After transcription, mRNAs undergo a series of intertwining processes to be finally translated into functional proteins. The 'post-transcriptional' regulation provides cells an extended option to fine-tune their proteomes. To meet the demands of complex organism development and the appropriate response to environmental stimuli, every step in these processes needs to be finely regulated and the deregulation could result in pathological condition(s). To understand the underlying molecular mechanism, we have been developing various genomic assays based on novel sequencing technology to study post-transcriptional regulation in the context of human diseases as well as during development and evolution.*

Global microRNA precursor profiling in mammalian cells

MicroRNAs (miRNAs) constitute an important class of small regulatory RNAs that are derived from distinct hairpin precursors (pre-miRNAs). In contrast to mature miRNAs, which have been characterized in numerous genome-wide studies of different organisms, research on global profiling of pre-miRNAs is limited. Using massive parallel sequencing, we have performed global characterization of both mouse mature and precursor miRNAs. Our analysis revealed new aspects of miRNA/pre-miRNA processing and modification, including eight Ago2-cleaved pre-miRNAs, new instances of miRNA editing and exclusively 5' tailed mirtrons. Furthermore, based on the sequences of both mature and precursor miRNAs, we developed a miRNA discovery pipeline, miRGrep, which does not rely on the availability of genome reference sequences. In addition to 239 known mouse pre-miRNAs, miRGrep predicted 41 novel ones with high confidence. Similar as known ones, the mature miRNAs derived from most of these novel loci showed both reduced abundance following Dicer knockdown and the binding with Argonaute2. Evaluation on data sets obtained from Caenorhabditis elegans and Caenorhabditis sp.11 demonstrated that miRGrep could be widely used for miRNA discovery in metazoans, especially in those without genome reference sequences.

Ultra-deep profiling of alternatively spliced Drosophila Dscam isoforms by circularization assisted multi-segment sequencing (CAMSeq)

The Drosophila melanogaster gene Dscam (Down syndrome cell adhesion molecule) can generate thousands of different ectodomains via mutual exclusive splicing of three large exon clusters. The isoform diversity plays a profound role in both neuronal wiring and pathogen recognition. However, the isoform expression pattern at the global level remained unexplored. Here, we developed a novel method, CAMSeq that allows for direct quantification of the alternatively spliced exon combinations from over hundreds of millions of Dscam transcripts in one sequencing run (Figure 1). With unprecedented sequencing depth, we detected a total of 18496 isoforms, out of 19008 theoretically possible combinations. Importantly, we demonstrated that alternative splicing between different clusters is independent. Moreover, the isoforms were expressed across a broad dynamic range, with significant bias in cell/tissue and developmental stage-specific patterns. Hitherto underappreciated, such bias can dramatically reduce the ability of neurons to display unique surface receptor codes. Therefore, the seemingly excessive diversity encoded in the Dscam locus might nevertheless be essential for a robust self and non-self discrimination in neurons.

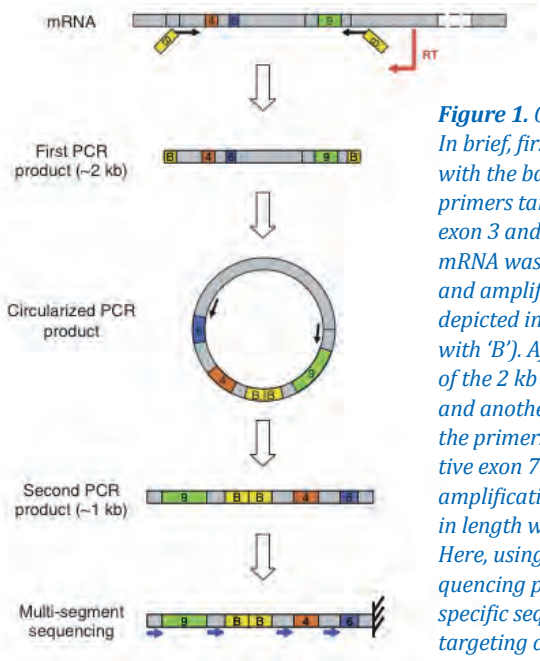


Figure 1. *Outline of CAMSeq. In brief, first, using RT–PCR with the barcode-indexed primers targeting constitutive exon 3 and exon 10, Dscam mRNA was reverse-transcribed and amplified (barcodes are depicted in yellow and marked with 'B'). After circularization of the 2 kb RT–PCR product and another round of PCR with the primers targeting constitutive exon 7 and exon 8, the amplification product of 1 kb in length was then sequenced. Here, using a modified sequencing procedure with four specific sequencing primers targeting constitutive exon 3, exon 5, exon 8, and exon 10, respectively (blue arrows), we obtained from every template DNA molecule four sequencing reads derived from exons 4, 6, 9, and barcode, respectively.*

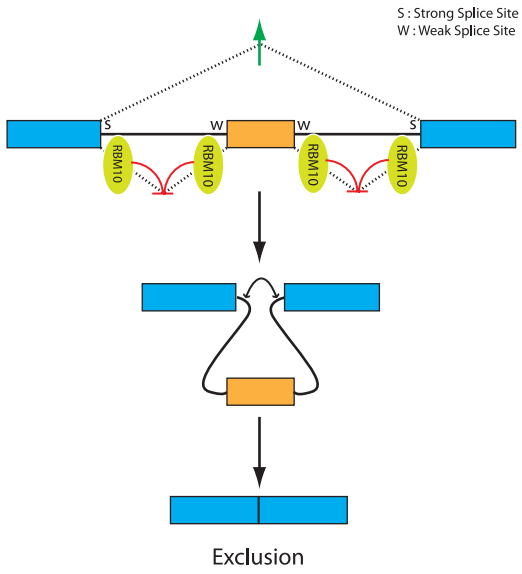


Figure 2. *Mechanistic model for RBM10-mediated splicing regulation. RBM10 binding in the vicinity of splicing sites might repress the splicing of flanking introns and delay the splicing choice, thereby facilitate the skipping of cassette exons flanked with relatively weaker splice sites.*

Integrative analysis of alternative splicing regulated by RBM10

RBM10 encodes an RNA binding protein. Mutations in RBM10 are known to cause multiple congenital anomaly syndrome in male humans, the TARP syndrome. However, the molecular function of RBM10 is unknown. Here we used PAR-CLIP to identify thousands of binding sites of RBM10 and observed significant RBM10–RNA interactions in the vicinity of splice sites. Computational analyses of binding sites as well as loss-of-function and gain-of-function experim ents provided evidence for the function of RBM10 in regulating exon skipping and suggested an underlying mechanistic model (Figure 2), which could be subsequently validated by minigene experiments. Furthermore, we demonstrated the splicing defects in a patient carrying an RBM10 mutation, which could be explained by disrupted function of RBM10 in splicing regulation. Overall, our study established RBM10 as an important regulator of alternative splicing, presented a mechanistic model for RBM10-mediated splicing regulation and provided a molecular link to understanding a human congenital disorder.

Systematic identification of genetic factors underlying congenital disorders

To expedite the molecular elucidation of genetic factors underlying rare diseases, we have established a high-throughput mutation screening approach by combining target region enrichment and next generation sequencing. Together with the clinical partners from Charite, we have succeeded in the identification of causative genetic defects implicated in a variety of congenital disorders.

Selected Publications

Wang, Y., Gogol-Döring, A., Hu, H., Fröhler, S., Ma, Y., Jens, M., Maaskola, J., Murakawa, Y., Quedenau, C., Landthaler, M., Kalscheuer, V., Wieczorek, D., Wang, Y., Hu, Y*. and Chen, W*: Integrative analysis revealed the molecular mechanism underlying RBM10-mediated splicing regulation. EMBO Mol Med. 2013 Sep;5(9):1431-42

Sun W, You X, Gogol-Döring A, He H, Kise Y, Sohn M, Chen T, Klebes A, Schmucker D*, Chen W*: Ultra-deep profiling of alternatively spliced Drosophila Dscam isoforms by circularization-assisted multi-segment sequencing. EMBO J. 2013 Jul 17;32(14):2029-38

Li N, You X, Chen T, Mackowiak SD, Friedländer MR, Weigt M, Du H, Gogol-Döring A, Chang Z, Dieterich C, Hu Y, Chen W: Global profiling of miRNAs and the hairpin precursors: insights into miRNA processing and novel miRNA discovery. Nucleic Acids Res. 2013 Apr 1;41(6):3619-34

Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J*, Chen W*, Selbach M* Global quantification of mammalian gene expression control Nature. 2011 May 19;473(7347):337-42

Adamidi C, Wang Y, Gruen D, Mastrobuoni G, You X, Tolle D, Dodt M, Mackowiak SD, Gogol-Doering A, Oenal P, Rybak A, Ross E, Alvarado AS, Kempa S*, Dieterich C*, Rajewsky N*, Chen W*. De novo assembly and validation of planaria transcriptome by massive parallel sequencing and shotgun proteomics. Genome Res. 2011 Jul;21(7):1193-200

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Photo: David Ausserhofer/MDC

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Metabolic Regulation

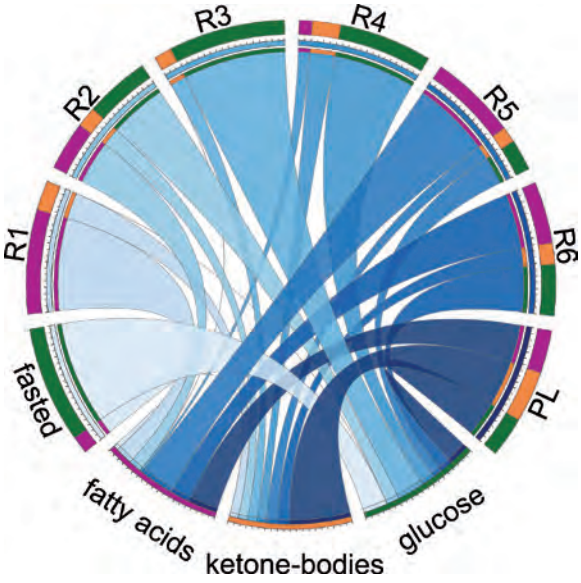
The central carbon metabolism (CCM) is the principal source of energy and building blocks for all cellular processes. Although the structure of this super-pathway is highly conserved in higher organisms, the regulation is rather complex, and appears at all levels of gene regulation - epigenetic, (post-) transcriptional (post-) translational and on an allosteric level. The structure of the regulatory and signaling network and the expression of alternative enzyme variants determine the metabolic state and flexibility. To gain insights in regulatory properties at a mechanistic level we apply metabolomics and proteomics techniques allowing the quantitative and dynamic measurement of the biochemical network in high resolution.

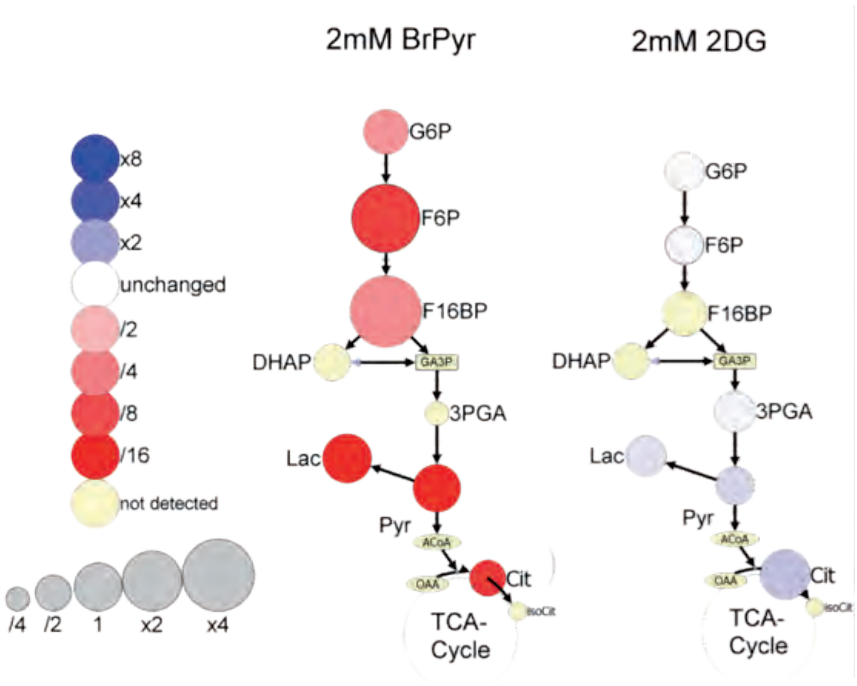
The interest of our group is twofold; on the one hand, we are interested in decoding the metabolic composition of the blood metabolome (in vivo) as it represents the overlay of the activity of all organs. On the other hand, we are studying metabolic regulatory processes at the cellular levels in molecular details (in vitro). The final goal is to merge these informations and to understand the consequences of a gene defect and its expression in metabolic signatures during the next years to come.

Decoding the blood metabolome

The blood summarizes all metabolic activities and cross talks between the individual organs. Due to specialized functions of the organs, e.g. sink and source relationships, it may be possible to relate specific metabolic patterns to the metabolic activity of organs. The liver as a central metabolic organ regulates the supply of fuel sources in the ground state and adapts the fuel supply during starvation or activity. In a case study we profiled the metabolome after sampling blood all ten minutes during a long distance run. The results about the association of the known fuel sources during that time course were visualized - we were able to follow a switch within the metabolic program 'online'.

Visualization of distribution of fuel sources during exercise in a human individual. *Blood samples were collected and analyzed by GC-MS based metabolomics. The circos-plot visualizes the three main energy sources and the time profile during a long distance run. Samples were taken all 10 min. The different phases of the exercise are fasted (short before exercise, R1-6 (round 1-6) PL post exercise.*





Visualization of the metabolic changes *within CCM after 15 min application of two glycolytic inhibitors (3-bromo pyruvate, 2-deoxyglucose). The size of the circles encode the x-fold change of the concentration and the color visualizes the change of stable isotope incorporation resulting from a three minutes pulse with $^{13}C_6$ glucose.*

Regulation of cellular metabolism

The intense crosstalk between regulatory networks and metabolism emerges as a new research focus. To analyze the dynamics of cellular metabolism we have established and modified GC-MS based metabolomics techniques and developed pulse stable isotope resolved metabolomics (pSRIM). Using this technique, we studied reprogramming of metabolism and synthetic lethal interactions in cancer cells (Liu et al. 2012, Dorr et al. 2013).

Metabolic reprogramming is a key step in oncogenic transformation including the activation of energy- and anabolic-metabolism. Thus, differentiated cells reenter the cell cycle and proliferate. Central to this is a strong up-regulation of glucose and glutamine consuming pathways. Ongoing from observations made already one century ago, the aerobic glycolysis also termed as 'Warburg effect', raised the questions for metabolic cancer therapies. Studying the mode of action of known anti-glycolytic metabolites revealed unexpected results. Using the pSIRM-technology we compared the anti-glycolytic effects of 2-deoxy glucose and 3-bromo pyruvate. 3-bromo pyruvate inhibits glycolysis very effectively but 2-deoxy glucose has only a minor influence on CCM. These results demonstrate that we have to carefully evaluate the current knowledge in order to design and test metabolic anti-cancer therapies.

In future, we aim to better understand the events of metabolic reprogramming during stem cell differentiation and oncogenesis. Specifically the relationship between metabolism and epigenetic regulation needs to be disentangled.

Selected Publications

Synthetic lethal metabolic targeting of cellular senescence in cancer therapy.
Doerr, J.R. and Yu, Y. and Milanovic, M. and Beuster, G. and Zasada, C. and Daebritz, J.H.M. and Lisec, J. and Lenze, D. and Gerhardt, A. and Schleicher, K. and Kratzat, S. and Purfuerst, B. and Walenta, S. and Mueller-Klieser, W. and Graeler, M. and Hummel, M. and Keller, U. and Buck, A.K. and Doerken, B. and Willmitzer, L. and Reimann, M. and Kempa, S. and Lee, S. and Schmitt, C.A. Nature 501 : 421-425. 19 September 2013

Deregulated MYC expression induces dependence upon AMPK-related kinase 5.
Liu, L. and Ulbrich, J. and Mueller, J. and Wuestefeld, T. and Aeberhard, L. and Kress, T.R. and Muthalagu, N. and Rycak, L. and Rudalska, R. and Moll, R. and Kempa, S*. and Zender, L. and Eilers, M. and Murphy, D.J. Nature 483 (7391): 608-612. 29 March 2012 (corr. for metabolomics/proteomics)

Proteome dynamics and early salt stress response of the photosynthetic organism Chlamydomonas reinhardtii.
Mastrobuoni, G. and Irgang, S. and Pietzke, M. and Wenzel, M. and Assmus, H.E. and Schulze, W.X. and Kempa, S. BMC Genomics 13 (1): 215. 31 May 2012

De novo assembly and validation of planaria transcriptome by massive parallel sequencing and shotgun proteomics.
Adamidi, C. and Wang, Y. and Gruen, D. and Mastrobuoni, G. and You, X. and Tolle, D. and Dodt, M. and Mackowiak, S.D. and Gogol-Doering, A. and Oenal, P. and Rybak, A. and Ross, E. and Alvarado, A.S. and Kempa, S*. and Dieterich, C. and Rajewsky, N. and Chen, W. Genome Research 21 (7): 1193-1200. July 2011 (*co-corresponding author)

Muscle-type creatine kinase interacts with central domains of the M-band proteins myomesin and M-protein.
Hornemann, T. and Kempa, S. and Himmel, M. and Hayess, K. and Fuerst, D.O. and Wallimann, T. Journal of Molecular Biology 332 (4): 877-887. 26 September 2003

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Photo: David Ausserhofer/MDC

Markus Landthaler

RNA Biology and Posttranscriptional Regulation

Our main interest is the understanding of posttranscriptional regulatory networks controlling gene expression in humans. Nascent RNA stably associates with RNA-binding proteins, RNA helicases and nucleases to form ribonucleoprotein complexes. These complexes play a key role in the regulation of spatial and temporal changes in protein synthesis by controlling transport, storage and translation of mRNAs. Deregulation and failed coordination of these mechanisms contribute to pathophysiologicial development and conditions. A prerequisite for a systems level understanding of posttranscriptional regulation is a transcriptome-wide high-resolution map of the RNA-protein contacts that allows us to study how these interactions control the fate of cytoplasmic RNA. To achieve this goal we use a novel crosslinking approaches (PAR-CLIP [Hafner & Landthaler et al. Cell 2010] and protein occupancy profiling on mRNA [Baltz & Munschauer et al. Mol Cell 2012) in combination with next-generation sequencing to identify functional RNA-protein interactions at single-nucleotide resolution.

Post-transcriptional regulation by RNA-binding proteins and microRNAs

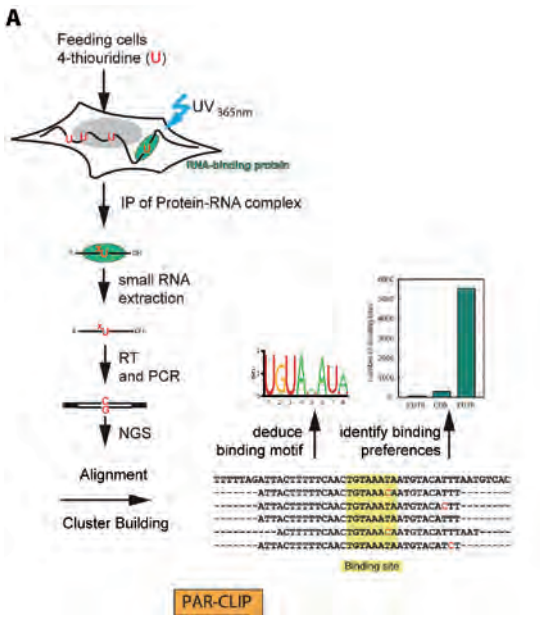
Yasuhiro Murakawa, Alexandra Vasile

Mammalian genomes encode several hundred RNA-binding proteins, each containing one or multiple domains able to recognize target mRNA in a sequenceand/or structure-dependent manner. The association of these proteins with transcripts regulates the biogenesis and translation of mRNA. For a large number of RNA-binding proteins the target mRNAs and their function in RNA metabolism are unknown, limiting our understanding of post-transcriptional regulatory processes.
In particular, we are interested in RNA-binding proteins that positively and negatively modulate the activity of microRNAs. By combining maps of functional RNA-protein interactions with cell-based and biochemical assays, we determine the dynamic assembly of RNA-binding proteins and microRNAs on their target mRNAs as well as the elements and mechanisms guiding mRNA maturation, localization, turnover and protein synthesis.

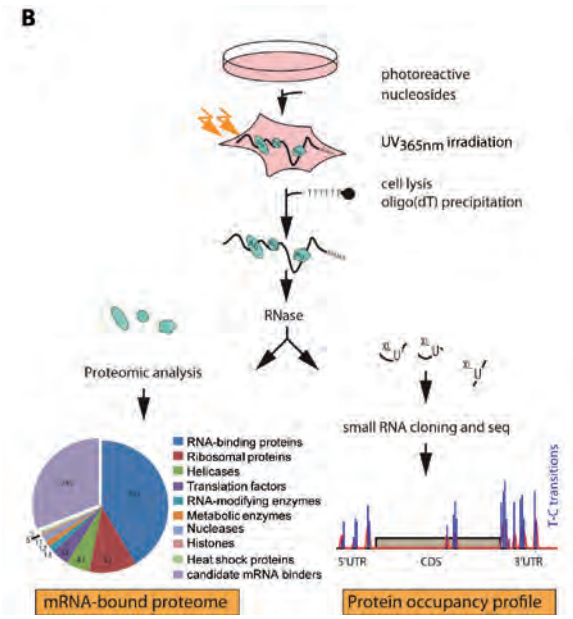
Specificity and function of RNA helicases

Lea Gregersen, Mathias Munschauer

RNA helicases are a family of highly conserved proteins that utilize NTP hydrolysis to unwind RNA structures and/or remodeling of ribonucleoprotein complexes. RNA helicases participate in all biological processes that involve RNA metabolism, including transcription, splicing and translation and have been implicated in disease states such as tumorgenesis and the replication of viruses. We are using our PAR-CLIP approach to define functional interactions of helicases, their helicase-inactive variants and RNA that are typically transient in nature. The identification of RNA target sites provides the foundation for biochemical and reverse genetic approaches to investigate the remodeling mechanism of ribonucleoprotein complexes by helicases. These studies give insights into the determinants of target RNA selection, functional interactions with other RNA-interacting proteins and the physiological role of RNA helicases.



A) PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation).



B) Identification of the mRNA-bound proteome and protein occupancy profiling on mRNA.

Global Changes in the mRNA-protein interactome upon stress and differentiation

Alexander Baltz, Miha Milek, Mathias Munschauer, Emanuel Wyler

We have developed a photoreactive nucleotide-enhanced UV crosslinking and oligo(dT) purification approach to identify the mRNA-bound proteome using quantitative proteomics and to display the protein occupancy on mRNA transcripts by next-generation sequencing. Application to a human embryonic kidney cell line identified close to 800 proteins. Nearly one-third were not previously annotated as RNA binding. Protein occupancy profiling provides a transcriptome-wide catalog of potential cis-regulatory regions on mammalian mRNAs and showed that large stretches in 3' UTRs can be contacted by the mRNA-bound proteome, with numerous putative binding sites in regions harboring disease-associated nucleotide polymorphisms. Our observations indicate the presence of a large number of mRNA binders with diverse molecular functions participating in combinatorial posttranscriptional gene-expression networks.

More recently we described a global comparison of protein occupancy profiles on mRNA transcriptomes from different cell types, and provided evidence for altered mRNA metabolism as a result of differential protein-mRNA contacts. Additionally, in this work we introduced a bioinformatics workflow for the analysis of protein occupancy profiling experiments. Our work demonstrated the value of protein occupancy profiling for assessing the cis-regulatory mRNA sequence space and its dynamics in growth, development and disease.

We use the described methods to monitor the dynamic changes of the mRNA-protein interactome to capture differentially binding proteins and occupied cis-regulatory mRNA regions as a consequence of intra- and extracellular signals. These data provide insights into the mechanisms that lead to changes in mRNA turnover and protein synthesis in response to stress and during differentiation.

Selected Publications

Gregersen LH, Schueler M, Munschauer M, Mastrobuoni G, Chen W, Kempa S, Dieterich C, Landthaler M. (2014) MOV10 is a 5' to 3' RNA helicase contributing to UPF1 mRNA target degradation by translocation along 3'UTRs. Molecular Cell 54, 1-13

Schueler M, Munschauer M, Gregersen LH, Finzel A, Loewer A, Chen W, **Landthaler M*,** Dieterich C*. (2014) Differential protein occupancy profiling of the mRNA transcriptome. Genome Biology 15:R15. (* equal contribution)

Graf R*, Munschauer M*, Mastrobuoni G, Mayr F, Heinemann U, Kempa S, Rajewsky N*, **Landthaler M***. (2013) Identification of LIN28B-bound mRNAs reveals features of target recognition and regulation. RNA Biology 10:1146-1159 (* equal contribution)

Memczak S, Jens M, Elefsinioti, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, **Landthaler M**, Kocks C, le Noble F, and Rajewsky N (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495:333-338

Baltz AG, Munschauer M, Schwanhausser B, Vasile A, Murakawa Y, Schueler M, Youngs N, Penfold-Brown D, Drew K, Milek M, Wyler E, Bonneau R, Selbach M, Dieterich C, and **Landthaler, M** (2012) The mRNA-Bound Proteome and Its Global Occupancy Profile on Protein-Coding Transcripts. Molecular Cell 46, 674-90

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Photo: David Ausserhofer/MDC

Alexander Loewer

Signaling Dynamics in Single Cells

Regulation of proliferation and differentiation is a fundamental challenge for multicellular organisms. Decisions between alternative cell fates are therefore tightly controlled by external and internal signals. Through genetics, genomics and proteomics, many molecular players that process these inputs have been identified, providing topological maps of most signaling networks. We are now challenged to understand how these networks act dynamically in living cells and how they intersect with each other to control the physiological response of a cell. As individual cells within a population often show distinct responses to the same signals depending on their internal state, we focus on the analysis of single cells and investigate the properties that unite them and the sources of variation that make them different. We use time-lapse microscopy of living cells to measure signaling dynamics with high temporal and spatial resolution and combine the resulting quantitative data with mathematical modeling to gain a predictive understanding of cellular decision processes.

P53 dynamics and function in health and disease

Elena Cristiano, Dhana Friedrich, Ana Finzel Perez

The tumor suppressor p53 is a central regulator of cellular proliferation. It is activated by cellular stress and controls target genes that mediate response programs such as cell cycle regulation, repair and apoptosis. In response to genotoxic stress, p53 acts as a sensitive signaling system that responds even to low amounts of DNA damage with a pulse of p53 protein accumulation in the nucleus. However, it was debated whether a defined threshold of damage exists for its activation. Using fluorescent reporters we developed a system for following in individual cells the number of damaged sites on DNA, the kinetics of damage repair and the p53 response (with Galit Lahav, Harvard Medical School). We found a large variation in the initial number of DNA break sites and the rate of repair between individual cells. Cells with higher number of breaks had higher probability of showing a p53 pulse. However, there was no distinct threshold for inducing a p53 response. Our results further suggest that the decision to activate p53 given a specific number of breaks is not entirely stochastic, but instead is influenced by both cell-intrinsic factors and previous exposure to DNA damage.

To understand the function of p53 signaling on a system's level, we identified and characterized relationships between atomic, molecular and cellular events that steer cell fate decisions. In collaboration with Phil Selenko (FMP Berlin) and Gary Daughdrill (University of South Florida), we showed that the extent to which a regulatory domain of p53 folds into an ordered structure defines the binding affinity to its major negative regulator Mdm2. By introducing single point mutations, we enhanced Mdm2 binding without affecting the remaining network configuration and demonstrated that this leads to altered cellular p53 dynamics, target gene expression and ultimately cell fate decisions. Our results provide evidence that the biophysical properties of p53 have been evolutionary selected to fine-tune the affinities of its many protein-protein interactions without compromising on binding specificity, thereby allowing this tumor suppressor to function as a hub in a central cellular signaling network.

To gain a better understanding of the p53 mediated cellular response, we investigate how p53 controls the temporal activation of its target genes. We developed reporter cell lines to simultaneously quantify p53 dynamics and expression of selected target genes.

We are now perturbing the network by introducing mutations, applying pharmacological interventions or altering interacting signaling pathways and determine how these changes affect the cellular response. This will allow us to better understand how modulating the p53 response during disease and therapy changes cell fate decisions and, potentially, clinical outcome.

Dynamics of the TGFβ pathway in single cells

Henriette Strasen, Derya Günes

The TGFb pathway interacts closely with the p53 network to induce a cytostatic response. We established reporter cell lines for central components of the TGFβ network and characterized pathway activation in individual cells in response to different inputs. One major goal is to identify the dominant feedback mechanisms that determine the long-term dynamic behavior of the network. To this end, we apply siRNA- or small molecule inhibitor-mediated perturbations of the signaling network and use mathematical modeling (in collaboration with Stefan Legewie, IMB Mainz) to gain a systematic understanding of the underlying signaling mechanisms.

Information flow in mammalian signal transduction pathways

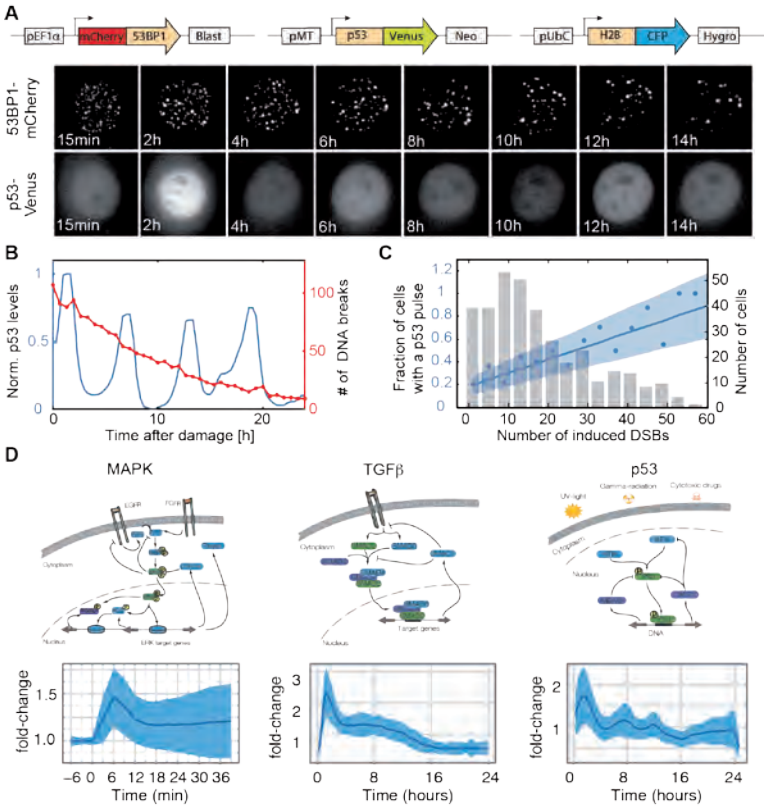
Ilias Nolis

Signal transduction networks relay information about external or internal inputs to the nucleus, and alter cellular behaviour by changing gene expression. As they exhibit striking similarities to typical communication systems, the powerful framework of information theory can be applied to better understand these systems. In collaboration with Nils Blüthgen (Charité Berlin), we systematically investigate the information transmission properties of the MAPK cascade and compare them to properties of other signalling systems such as the p53 or TGFβ pathways. We focus on understanding how information about ligand concentration and ligand type is encoded and decoded in the response of the pathways, how large the capacity of the pathways is as information transmitting channels and which design principles in the systems attenuate noise and therefore increase the amount of information that can be transmitted.

Genomic engineering

Caibin Sheng

In recent years, powerful new tools emerged to engineer endogenous gene loci. We are optimizing the CRISPR/Cas9 system to enable efficient insertion of fluorescent protein fusions at genes encoding critical mediators of cellular signaling, allowing us to generate advanced reporter cell lines with high throughput.



A) *A fluorescent reporter cell line allows simultaneous measurement of DNA double strand breaks (53BP1-mCherry) and p53 dynamics in individual living cells. DNA damage was induced by γ-irradiation, followed by time-lapse microscopy for 24h.* ***B)*** *Using automated image analysis, both the number of DNA breaks and p53 levels are quantified over time. P53 protein accumulates in uniform pulses during repair of the damage.* ***C)*** *There is no defined threshold of DNA breaks for inducing a p53 pulse, however, cells with higher number of breaks have higher probability of showing a response (from Loewer et al. (2013) BMC Biol).* ***D)*** *Using fluorescent reporters and live cell imaging, we quantify the dynamics of various signaling pathways in individual living cells to understand how the topology of the underlying molecular networks determine the outcome of cellular signaling processes.*

Selected Publications

Loewer, A.#, Karanam, K., Mock, C., Lahav, G.# (2013). The p53 response in single cells is linearly correlated to the number of DNA breaks without a distinct threshold. BMC Biol 11:114 #corresponding author

Karanam, K., Kafri, R., Loewer, A., Lahav, G. (2012). Quantitative Live Cell Imaging Reveals a Gradual Shift between DNA Repair Mechanisms and a Maximal Use of HR in Mid S Phase. Mol. Cell 47(2): 320-9

Purvis, JE., Karhohs, KW., Mock, C., Batchelor, E., Loewer, A., Lahav, G. (2012). p53 dynamics control cell fate. Science 336(6087): 1440-4

Orth, JD., Loewer, A., Lahav, G., Mitchison, TJ. (2012). Prolonged mitotic arrest triggers partial activation of apoptosis, resulting in DNA damage and p53 induction. Mol Biol Cell. 23(4): 567-76

Loewer, A., Batchelor, E., Gaglia, G., Lahav, G. (2010). Basal dynamics of p53 reveals transcriptionally attenuated pulses in cycling cells. Cell 142(1): 89-100

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Photo: David Ausserhofer/MDC

Benedikt Obermayer

Evolutionary Modeling and Inference in Computational Systems Biology

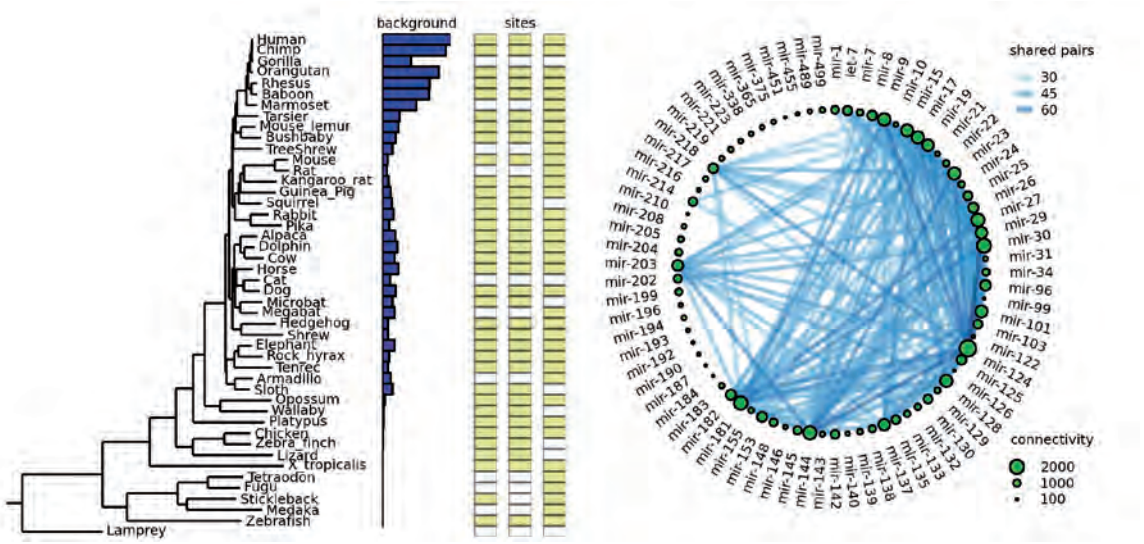
Efforts to decode genomic functions benefit from understanding the constraints on genome evolution. These constraints result from different processes and work on different scales. For instance, biochemical limitations of the cellular machinery drive the evolution of robust control strategies. On a large scale, regulatory dependencies between genomic elements couple their respective evolutionary dynamics. Importantly, different types of evolutionary constraints leave different types of sequence fingerprints. However, these signals need to be distinguished from a large background of more or less random sequence variation. Our approach to detect and organize informative evolutionary signals combines computational tools and modeling techniques from bioinformatics and statistical physics. With our methods, we address a variety of questions, from the accuracy of transcription and translation reactions to high-level inference of post-transcriptional regulatory networks and the detection of novel coding regions in the genome.

Modeling polymerization errors and control strategies

Many biological functions across all domains of life fundamentally depend on template-directed polymerization processes. Prime examples are DNA replication, RNA transcription and protein translation. Physical constraints limit the accuracy of these reactions leading to occasional misincorporations. These errors can be tolerated when rare, and sometimes they even serve as a source for evolutionary innovations. However, in general their consequences are largely detrimental, which led to the evolution of error-control strategies on the one hand and the evolution of a substantially increased error tolerance on the other. Our previous work was concerned with quantifying the extent to which simple non-enzymatic *in vitro* systems are constrained by the thermodynamics of RNA and DNA duplex formation. Our observations suggested simple control strategies that could be advantageous in hypothetical prebiotic (enzyme-free) environments. Current work explores technological applications of these ideas that could help to improve the fidelity of PCR reactions. Together with the lab of Michael Gotthardt, we also study physiological implications of the evolved balance between fidelity and robustness in the context of cardiovascular gene expression.

Evolution of post-transcriptional regulatory networks

Post-transcriptional gene regulation is a major determinant of cellular gene expression. Messenger RNAs (mRNAs) are under tight control by various regulatory factors during their entire life cycle. Among these factors are microRNAs (miRNAs), acting on target sites located in the 3' untranslated regions (UTRs) of mRNAs. Globally, this mode of post-transcriptional gene regulation has been found to depend less on individual regulatory links between a single miRNA and a single target site. More often, it is conveyed through the collective and concerted regulation by multiple miRNAs of entire target gene sets. Although individual target sites often have minor regulatory or phenotypic consequences and are therefore typically not widely conserved, the collective function of the entire network appears to remain much more stable during the course of vertebrate genome evolution. This implies that individual sites do not evolve independently of each other. We have developed computational methods to detect such non-



Evolutionary correlations in the miRNA regulatory network: *preferential conservation of miRNA target sites is evaluated by means of a phylogenetic background model trained on 3'UTR conservation in vertebrate whole-genome alignments. Comparing site pairs, significant similarity of dissimilarity of their conservation patterns is then detected as positive or negative evolutionary correlation, respectively. This analysis suggests that miRNAs with many positively correlated site pairs in the same 3'UTR share common functions in the regulatory network.*

independent evolution (i.e., evolutionary correlations) in the vertebrate miRNA regulatory network. Our results show that combinatorial regulation via an ensemble of multiple target sites in the same 3'UTR is not only apparent from consistent co-occurrence of such target sites, but also from their co-conservation. Further, we find that the conservation signatures for the coordinated targeting of protein complexes differs from those for the regulation of signaling pathways, possibly suggesting different control strategies. Our approach combines phylogenetic modeling with ideas from statistical physics, and we subsequently extended it in order to distinguish direct and indirect evolutionary correlations. One application of these inference techniques has recently proven very useful in aiding computational protein folding with distance constraints derived from correlated substitutions in protein sequence alignments. By our theoretical analysis and with our generalized methods we aim to transfer these techniques to other biological contexts.

Small open reading frames coding for conserved micropeptides

(PhD student: Sebastian Mackowiak)

The coding potential of the genomes of most model organisms has been thought to be comprehensively explored. Hence, the research focus recently often shifted to the discovery and characterization of putatively non-coding mRNA transcripts. However, traditional methods for discovery of functional open reading frames (ORFs) relied on preconceptions about typical ORF characteristics. These have recently been shown to be rather incomplete with the discovery of functional micropeptides in various animals. Such peptides are not produced by cleaving larger precursors but translated from small ORFs that can be as short as 11 codons and are often hosted by transcripts so far annotated as non-coding. The abundance of micropeptides and their genomic features and biological functions remain largely unexplored. We develop and use computational tools to detect conserved small open reading frames in multiple species alignments for a number of different model organisms. We collaborate with the lab of Antonio Giraldez (Yale) to combine our computational pipeline with experimental detection of translated ORFs via ribosome footprinting in zebrafish. This revealed that numerous widely conserved small ORFs are translated during zebrafish development, suggesting that they give rise to functional products. We further work with several labs on the MDC campus to identify and characterize micropeptides in other model organisms via mass spectrometry, by means of structural analysis, and via genetics.

Selected Publications

Bazzini A.A.; Johnstone T.G.; Christiano R.; Mackowiak S.D.; Obermayer B.; Fleming E.S.; Vejnar C.E.; Lee M.T.; Rajewsky N.; Walther T.C.; Giraldez A.J. Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation The EMBO journal (doi: 10.1002/embj.201488411; published online 2014-04-04)

Leu K.; Kervio E.; Obermayer B.; Turk-MacLeod R.M.; Yuan C.; Luevano J.M.; Chen E.; Gerland U.; Richert C.; Chen I.A. Cascade of reduced speed and accuracy after errors in enzyme-free copying of nucleic acid sequences Journal of the American Chemical Society 135 (1): 354-366 (2013-01-09)

Leu K.; Obermayer B.; Rajamani S.; Gerland U.; Chen I.A. The prebiotic evolutionary advantage of transferring genetic information from RNA to DNA Nucleic Acids Research 39 (18): 8135-8147 (2011-10)

Obermayer B.; Krammer H.; Braun D.; Gerland U. Emergence of information transmission in a prebiotic RNA reactor Physical Review Letters 107 (1): 018101 (2011-07-01)

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Photo: David Ausserhofer/MDC

Uwe Ohler

Computational Regulatory Genomics

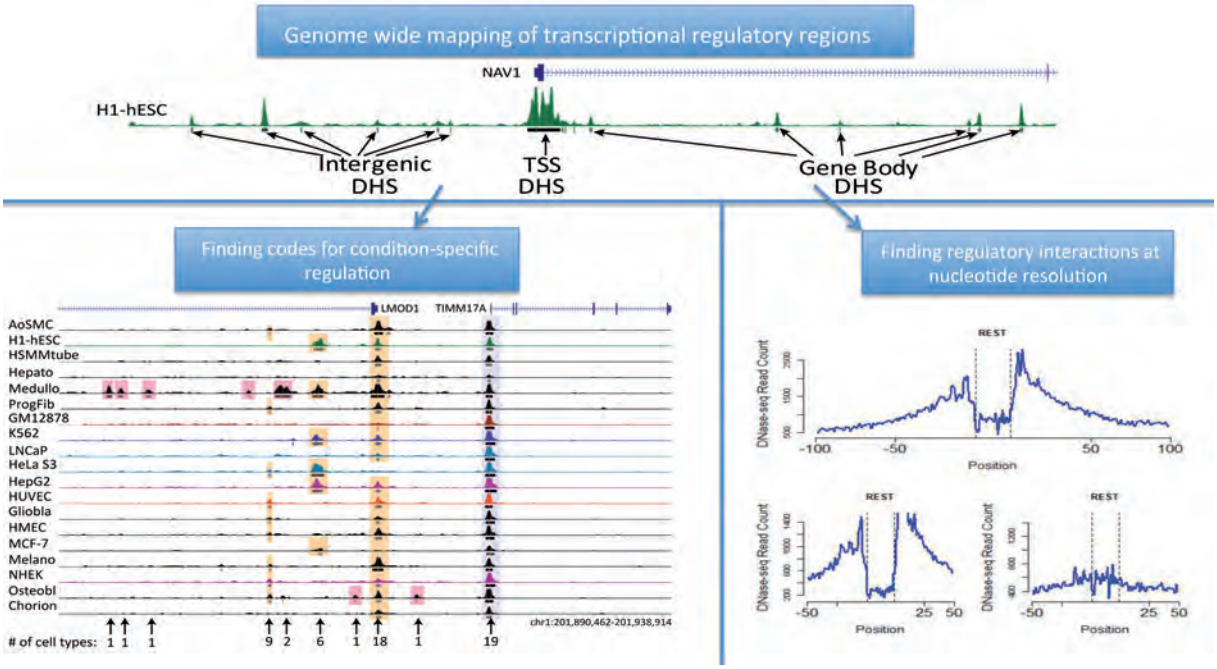
The expression of genes is a multi-step process that is tightly controlled on several levels — a large number of protein and RNA factors and DNA and RNA sequence elements enable the precise regulation of interacting gene products. Our lab develops and uses computational approaches to understand the biology of gene regulation in eukaryotic organisms.
According to our current understanding, complexity in higher organisms is not achieved by a more complex repertoire of parts, i. e. genes, but instead by the more complex regulation of the parts. It is a key challenge to decipher these complex networks of players and interactions, and to move biology from case studies to an integrated, global approach. Large initiatives such as the ENCODE consortium have set out to map all functional elements in the human genome, and in consequence, computational biology has become indispensable to analyze and ultimately make sense of large-scale data sets that look at the phenomenon of gene regulation from different angles. Our long term goal is to investigate how regulatory networks enable the correct development of complex organisms, with their multitude of cell types that carry out different functions despite the same genome.

Computational Biology and Gene Regulation

Understanding gene regulation emerged as a crucial challenge after the initial sequencing of complex eukaryotic genomes, and misregulation of gene expression is at the heart of many developmental defects and diseases. In this context, the particular strength of a multi-disciplinary group like ours lies in the combination of genomics-oriented data generation and analysis with computational modeling. We frequently frame questions as classification problems and use machine learning approaches to make testable predictions. These predictions are often validated experimentally, either by us or our friends and collaborators. Computational techniques range from simple (sparse) classifiers to complex approaches such as graphical models and Bayesian (non-parametric) statistics, to predict, annotate and analyze regulatory regions and the encoded expression patterns at a genome-wide scale. At the same time, inspection of model parameters frequently allows us to draw conclusions, or at least make specific suggestions, about the importance of particular biological features.

Transcriptional control: from proximal initiation to distal regulation

In eukaryotes, transcriptional regulatory elements are located in different regions relative to the gene they control (Figure 1). The basal or core promoter, a ~100 base pair long region centered on the transcription start site (TSS), has been traditionally assumed to perform the function to recruit the basal transcription complex to the correct genomic location, and thus to largely share the same sequence patterns across all genes. In contrast, distal non-coding regulatory regions such as enhancers contain a combination of sequence patterns recognized by specific transcription factors (TFs) and thus convey condition-spe-



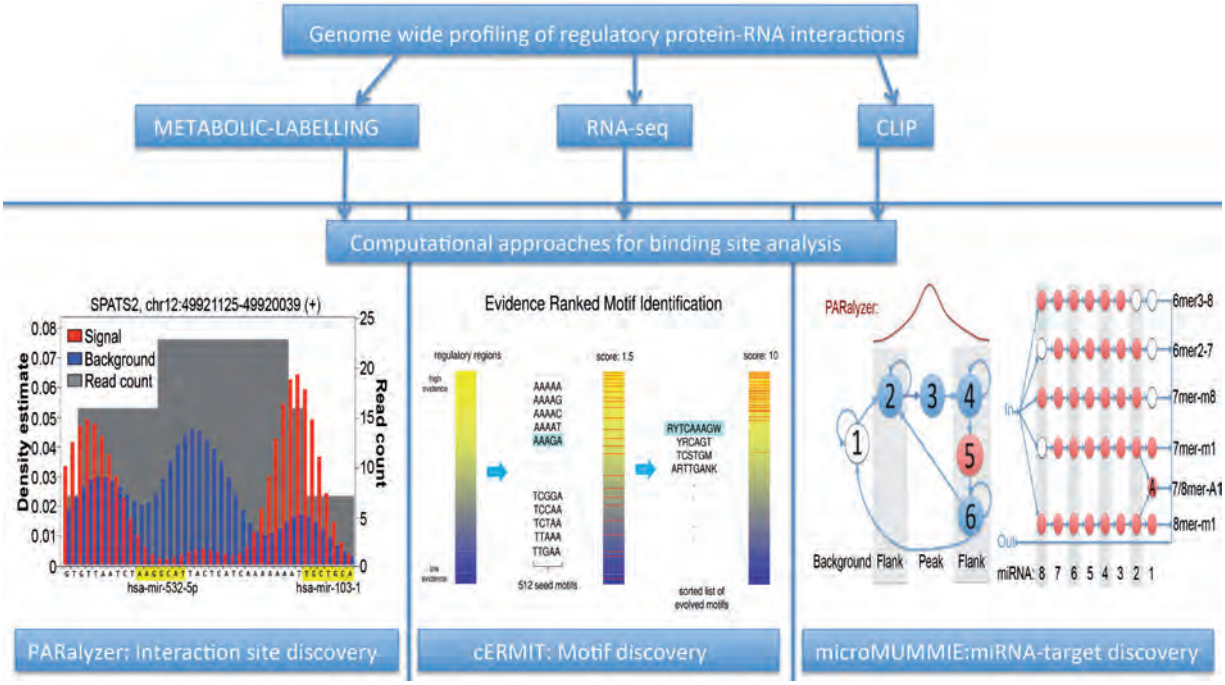
Modeling of transcriptional regulation. *A number of different deep sequencing protocols make it possible to identify putative control regions within the large space of non-coding sequence in the human genome. As example, regions of open chromatin that are accessible to regulatory factors can be identified at a genomic scale via DNase-seq. (Top) In this way, both the proximal promoters around transcription start sites of genes, as well as distal regulatory regions, can be identified. (Left) A subset of open chromatin regions are highly condition specific; knowing the location of these regions makes it possible to identify underlying sequence features and candidate regulators of specific expression patterns. (Right) At high resolution, DNase-seq data exhibits characteristic "footprints" at the sequence motifs that a transcription factor binds to. Using computational approaches, we can identify the subset of candidate sites that are occupied in a specific condition.*

cific activity (Ohler and Wassarman, Development 2010).

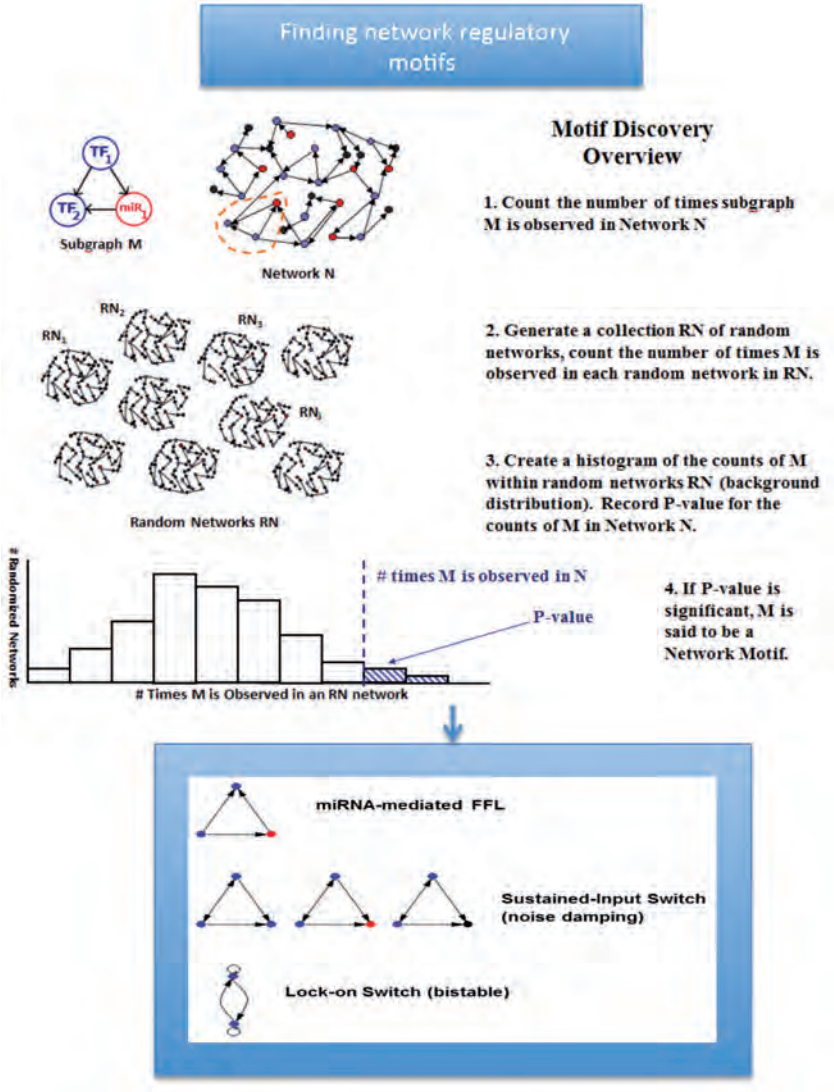
We have made particularly significant contributions towards an increased understanding of the core promoter as "model system" for complex regulatory regions. Our studies showed that core promoters do not show a single stereotypic arrangement and serve different functions (Rach et al, Genome Biol 2009). This is reflected in different initiation patterns, which have become apparent via new sequencing protocols that generate libraries of millions of short transcripts pinpointing the locations of transcription initiation (Ni et al, Nature Methods 2010). This diversity is observed across all eukaryotes; different types of core promoters distinguish themselves not just by sequence but also by conserved chromatin features (Rach et al, PLoS Genetics 2011). Eukaryotes thus have some species-specific sequence features but exhibit widely conserved chromatin patterns for different expression programs at the level of basal initiation (housekeeping genes, developmental regulators, translation machinery, germline, etc). In ongoing work, we examine connections of transcription initiation with pervasive non-coding/antisense transcription that has emerged as a feature of mammalian promoters.

In complementary work, we have made progress to decode cell type specific expression by integrating heterogeneous data sources from sequence, occupancy, expression and chromatin features. For a long time, the large non-coding genomic sequence space of higher eukaryotes has posed severe limitations as it made it difficult to pinpoint the short and degenerate sequence elements that are targeted by TFs, and to distinguish background sequence variation from changes with functional consequences. Several recent genomic approaches now provide "global regulatory profiles", which reflect where regulatory factors targeted specific regions andsites in DNA or RNA.

Specifically, DNaseI hypersensitive sites provide coarse-resolution maps of nucleosome-free sites or "open chromatin", which are universal hallmarks of all types of transcriptional regulatory elements. DHS site mapping has been widely used to identify regulatory elements that control gene transcription, and has been implemented in genome-wide protocols (Figure 1). In an ENCODE companion paper, we used DNase-seq open chromatin data from diverse cell types to build classifiers that provide a successful baseline for the prediction of expression from sequence features (Natarajan et al, Genome Research 2012). The resulting models contained factors with known as well as testable new functions, and illustrated the game-changing nature of genome-wide annotations of distal regulatory regions for decoding expression patterns. We



Computational approaches for investigating regulatory protein-RNA interactions. *Recent sequencing protocols make it possible to quantify levels of transcripts as well as to identify how they are regulated at the RNA level, and we have developed a number of computational methods for appropriate genome-wide analyses. (Left) Cross-linking and immunoprecipitation data can be used to identify specific locations on transcripts to which regulators are binding. (Middle) Motif finding algorithms can identify the prevalent sequence features present at the binding sites. (Right) Integrating multiple data types, such as sequence, binding, and conservation, allows for highly specific predictions of regulatory events such as targeting by microRNAs.*



Finding regulatory network motifs. *To study how transcription factors and microRNAs together control target genes within regulatory networks, we have developed a permutation strategy that enables the identification of significant "network motifs", i.e. preferential subnetwork structures that involve both TFs and miRNAs. Across different organisms, this algorithm identified three main types of such motifs.*

are now investigating models for DNase-seq data at nucleotide resolution, to make it possible to pinpointing the individual TF binding events within regulatory regions.

RNA regulation: identifying target sites of regulatory RNAs and proteins

The last decade has seen an outstanding increase in appreciation of the role of expression regulation at the RNA level and its importance for the correct function of virtually all biological processes. RNA-binding proteins and small, regulatory RNAs such as microRNAs (miRNAs) recognize specific sites in target RNA transcripts and influence their fate. The field has made dramatic advances through the invention of new deep transcriptome sequencing protocols that make it possible to quantify the levels of protein-coding messenger-RNAs and non-coding RNAs at different stages of post-transcriptional regulation (Figure 2). We are now also able to map the precise target sites of post-transcriptional regulators (including RNA-binding proteins and regulatory, non-coding RNAs) via cross-linking followed by deep sequencing.

These new data types pose intriguing computational challenges. Over the past few years, we have implemented specific new approaches for reliable, high-resolution identification of binding sites, as well as for follow-up prediction of regulatory sequence motifs, including miRNA target sites as a special case (Majoros et al, Nature Methods 2013). These approaches have been used in multiple genomic studies with collaborators to identify the genes regulated by disease-implicated RNA binding proteins such as the Fragile X mental retardation protein family (Ascano et al, Nature 2012) or the cellular targets of human viral miRNAs (Gottwein et al, Cell Host & Microbe 2011).

Putting the pieces together: networks involving different regulatory mechanisms

Our work on DNA and RNA-based regulation converges in projects where we aim to dissect the quantitative contributions of individual regulatory layers, and how they need to work together to define correct expression at different scales (e.g. during predefined development as compared to rapid responses to a changing environment). In a recent example, we have identified significant network motifs involving regulation of target genes by both TFs and miRNAs. For this aim, we needed to develop a new network motif identification algorithm which properly accounts for factors at different levels of gene expression (Figure 3). Across different organisms, we identified a subset of conserved network motifs, such as the frequent co-regulation of a TF Z by TF X and miRNA Y, where Y also targets Z (Megraw et al, Genome Biology 2013). This static network analysis will be complemented by dynamic gene expression data to quantify the function of different network motifs in different conditions.

The group moved from Duke University in late 2012. The described work had the support from a number of excellent colleagues, including Pavel Tomancak (MPI Dresden); James Kadonaga (UC San Diego); David Wassarman (University of Wisconsin); Tom Tuschl (Rockefeller University); Jun Zhu (NHLBI); Philip Benfey, Blanche Capel, Greg Crawford, Jack Keene (Duke University). We are now looking forward to extending these collaborations to the new possibilities at BIMSB and MDC.

Selected Publications

M Megraw, S Mukherjee, U Ohler. Sustained-input switches for transcription factors and microRNAs are central building blocks of eukaryotic gene circuits. Genome Biol 14:R85, 2013.

M Ascano, N Mukherjee, P Bandaru, JB Miller, JD Nusbaum, DL Corcoran, C Langlois, M Munschauer, S Dewell, M Hafner, Z Williams, U Ohler, T Tuschl. FMRP targets distinct mRNA sequence elements to regulate protein expression. Nature 492:382-386, 2012

WH Majoros, P Lekprasert, N Mukherjee, RL Skalsky, DL Corcoran, BR Cullen, U Ohler. MicroRNA target site identification by integrating sequence and binding information. Nature Methods 10:630-633, 2013

A Natarajan, GG Yardimci, NC Sheffield, GE Crawford, U Ohler. Predicting Cell-Type Specific Gene Expression from Regions of Open Chromatin. Genome Res 22:1711-1722, 2012.

EA Rach, DR Winter, AM Benjamin, DL Corcoran, T Ni, J Zhu, U Ohler. Transcription initiation patterns indicate divergent strategies for gene regulation at the chromatin level. PLoS Genet. 7:e1001274, 2011.

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Photo: David Ausserhofer/MDC

Ana Pombo

Epigenetic Regulation and Chromatin Architecture

Our laboratory aims to understand mechanisms of gene expression which act at multiple levels, from the local action of transcription factors, to long-range chromatin looping events that connect regulatory DNA sequences with the genes they regulate, to how whole chromosomes are positioned within cell nuclei (Figure 1).

Binding of transcription factors at gene promoters helps recruit chromatin remodelers and the transcription machinery to transcription sites. Upon recruitment, RNA polymerases either remain primed for subsequent activation (e.g. after environmental stress, during differentiation or disease), or directly transcribe the DNA template into RNA molecules, which code for proteins or for structural RNAs.

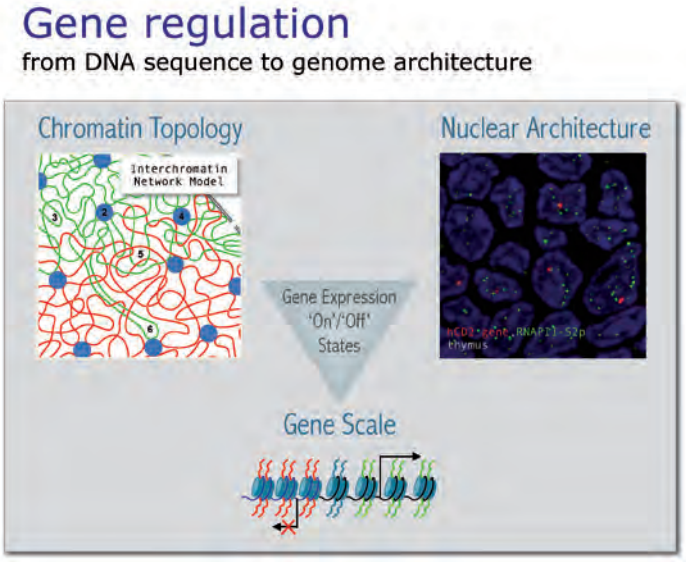
An increasingly important aspect of gene regulation are the mechanisms by which distant regulatory DNA sequences physically interact with gene promoters to recruit or activate RNA polymerase II (RNAPII), the enzyme responsible for transcribing protein-coding genes. Much is known about the transcription cycle of protein-coding genes, which entails a fascinating and highly coordinated sequence of processes that promote nascent transcript maturation concomitant with transcription. As the nascent RNA emerges from the polymerase, it is capped on its 5' end. Later, as the polymerase progresses through the coding region, introns are spliced from the nascent transcript. Upon transcription termination, addition of a protective polyA tail to the transcript's 3' end still occurs on chromatin. These co-transcriptional events are mediated by exquisite post-translational modifications of the RNAPII enzyme itself, which coordinate the timely recruitment of appropriate machineries at each stage of the transcription cycle. Chromatin modification of histone tails are also often coordinated by RNAPII itself, marking active promoters and their coding regions for further cycles of transcription.

Mechanisms of RNAPII and Polycomb regulation in pluripotent and differentiated cells

João Dias, Ana Miguel Fernandes, Carmelo Ferrai, Alexander Kukalev, Kelly Morris, Kedar Natarajan, Tiago Rito, Marisa Saponaro, Elena Torlai-Triglia

Appropriate recruitment of the RNA processing machinery and chromatin remodelers to chromatin is mediated by post-translational modification of the C-terminal domain (CTD) of the largest RNAPII subunit, Rpb1 (Brookes & Pombo 2009, EMBO Rep. 10, 1213). The CTD is an unusual disordered domain composed of the heptapeptide consensus sequence, Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7, repeated 52 times in mammals. All aminoacids are targets for post-translational modifications, of which we know most about Serine phosphorylation.

Our main aim is to understand the interplay between gene regulation and genome architecture, with the goal of defining rules and principles of genome function in mammals. We study local chromatin states using genome-wide methods to profile the occupancy of RNA polymerase II and epigenetic regulators, such as Polycomb repressor complexes. This information is used to interpret 3D chromatin folding, and to explore the role of gene positioning in the cell nucleus in gene expression



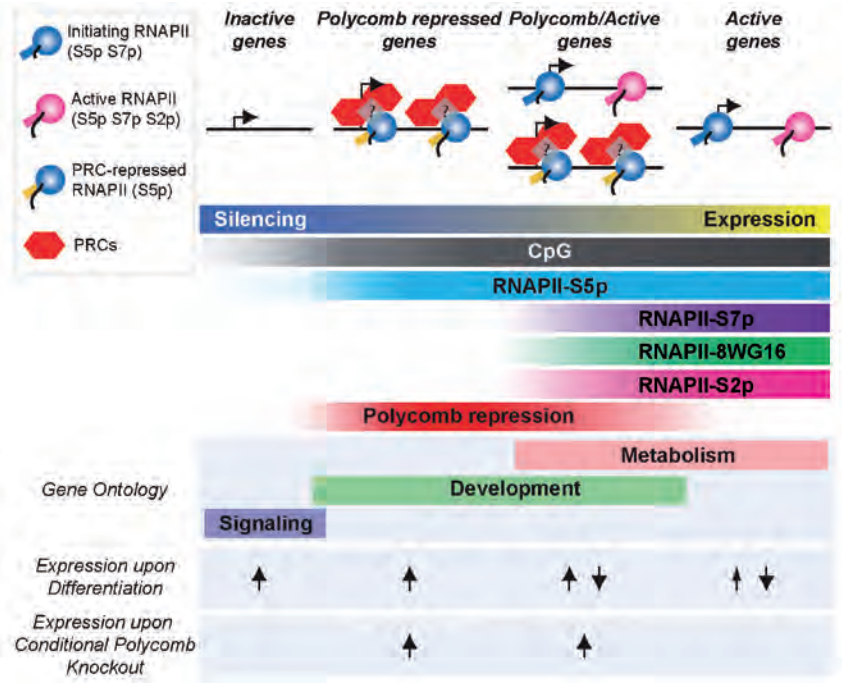
After recruitment of unphosphorylated RNAPII to promoters, the CTD becomes phosphorylated on Ser5 residues (S5p), followed by sequential phosphorylation on Ser7 (S7p) and Ser2 (S2p) in the transition to elongation. S5p recruits the capping enzyme and H3K4 histone methyltransferases (HMTs), which mark active promoters. S7p is thought to promote the transition to elongation, whereas S2p recruits both H3K36 HMT, to mark coding regions, and the splicing and polyadenylation machineries. Our unbiased mass spectrometry analyses of RNAPII complexes in mitosis (Moeller et al. 2012; in collaboration with M. Selbach) and in association with chromatin (ongoing work) identify a growing number of nuclear activities coordinated with RNAPII, many of which with roles in disease.

Regulation of RNAPII by phosphorylation extends beyond the changes associated with active transcription cycles. Phosphorylated RNAPII has been reported at inducible genes, before activation, e.g. heat-shock. Our group has identified a novel poised form of RNAPII at silent developmental regulator genes in mouse ES cells, which are repressed by Polycomb complexes, but become activated through early differentiation and cell commitment (Stock et al. 2007, Nature Cell Biol. 9, 1428). Two major Polycomb Repressor Complexes, PRC1 and PRC2, are important for maintaining the poised state of primed developmental regulator genes. Polycomb complexes modify histone tails; PRC2 contains Ezh2, which methylates H3K27, and PRC1 contains Ring1A and Ring1B, which monoubiquitinylate H2AK119, but the mechanisms of repression are not well understood.

In 2012, we showed that Polycomb and RNAPII co-associate on chromatin at developmental regulator genes (Brookes et al. 2012). We used chromatin immunoprecipitation (ChIP) coupled with next generation sequencing, to map the phosphorylation state of RNAPII and Polycomb occupancy across the genome (Figure 2). Surprisingly, not only did we find RNAPII at developmental regulator genes under Polycomb repression, but its presence was positively correlated with the amount and occupancy of Polycomb. To characterize the extent of transcriptional activity at poised genes, which are targets of Polycomb repression, we are analyzing chromatin-bound RNAs using genome-wide approaches, and CAGE-seq data (the latter in collaboration with FANTOM5).

Our unbiased genome-wide analyses of RNAPII and Polycomb occupancy also identified novel gene targets of Polycomb regulation in mouse ES cells, which were more unexpected (Brookes et al. 2012). In particular, we found a cohort of genes which are active in ES cells but whose expression is dampened by Polycomb repression (Polycomb-Active genes; Figure 2). This group of genes includes many genes important for cell cycle, cancer, signaling and energy metabolism. Our current studies aim to better understand their regulation in the transition from pluripotency to differentiated cellular states.

To probe for the dynamic changes in polymerase and chromatin conformation that accompany re-programming of gene expression during cell commitment, we have extended our analyses to neural progenitor



Model of RNA polymerase II (RNAPII) and Polycomb regulatory states at Active and Repressed genes in mouse ES cells (Brookes et al. 2012 Cell Stem Cell). Active genes are associated with RNAPII-S5p,S7p during initiation, with S5p,S7p,S2p during elongation, and with productive expression but no Polycomb association. Inactive genes are not associated with RNAPII or Polycomb. Polycomb Repressed genes are not expressed, have high CpG content, high RNAPII-S5p^{+}S7p^{-}S2p^{-} and Polycomb recruitment. An unexpected cohort of genes, which we call Polycomb-Active, show a mixture of Active and Polycomb repressed chromatin states. They are expressed at the mRNA and protein levels, yet they are up-regulated upon Polycomb knockout. These genes display different configurations at independent gene copies in the same or different cells across the ES cell population. Some alleles are repressed and associated with Polycomb and RNAPII-S5p with similar properties to PRC repressed genes. Other alleles are active and associate with active RNAPII complexes characterized by the presence of S5p and S2p (pink) but not Polycomb.

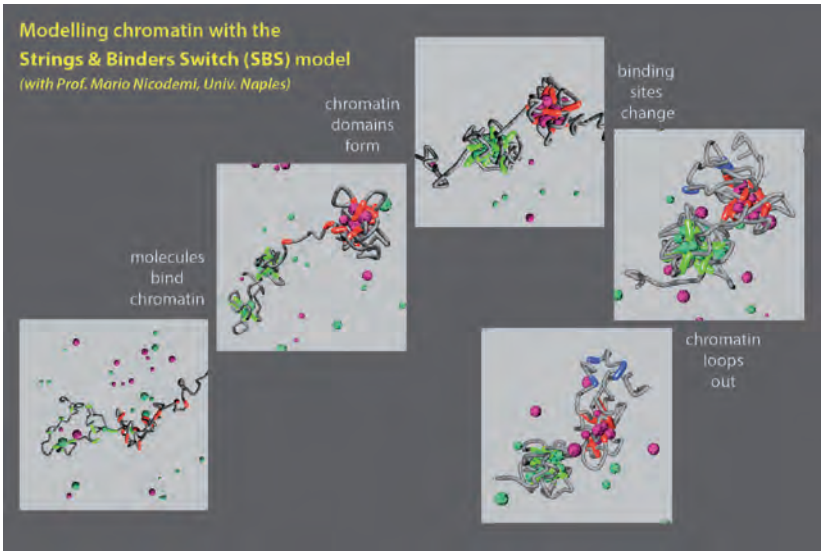
cells and differentiated neurons (co-funded by BBSRC and MRC, UK) and to cardiac lineages (co-funded by MRC-BHF, UK). Bioinformatics analyses are ongoing to further understand how Polycomb repression acts to establish poised states of RNAPII occupancy at genes with roles in pluripotency and differentiation.

Genome architecture: roles of RNAPII and Polycomb in 3D chromatin folding

Mariano Barbieri, Robert Beagrie, Inês de Castro, Dorothee Krämer, Liron Mark Lavitas, Markus Schueler

Long-range gene regulation works through chromatin looping mechanisms that bring together the promoters of genes with distant regulatory regions (e.g. enhancers). During this contact, mediated by transcription factors and other chromatin associated components, the transcriptional machinery is delivered to the gene, which acts as the starting point for the synthesis of transcripts. Genes that regulate early development are often expressed at different stages of differentiation and different cell types, and are accordingly regulated through specific interactions with different regulatory regions. In addition to contacts with their respective enhancers (which can be located up to 1 Mbp from the gene promoter), genes also co-associate with other genes in 3D space. Our group has shown that Active and Polycomb-repressed genes associate, respectively, with 'Active transcription factories' (containing RNAPII-S5p, S2p) and with 'Poised transcription factories' (containing RNAPII-S5p and Polycomb; Ferrai et al. 2010 PLoS Biol.; Brookes et al. 2012 Cell Stem Cell), suggested that such associations establish specific chromatin topologies that are important for silencing in the primed state, or for robust activation upon induction.

We currently use polymer physics modeling to study the mechanisms underlying complex chromatin folding patterns associated



Using the String & Binders Switch (SBS) model (Barbieri et al. 2012), we can study how epigenetic information coating mammalian chromosomes can fold into increasingly higher levels of chromatin architecture. Using computer simulations, we can test how chromatin factors that bind to their specialized sites along the DNA molecule can promote higher order chromatin folding. Over time (moving from left to right and down in this picture), different floating factors (red and green) bind their respective sites on the DNA to create loops. As more proteins attach, loops become wound closer together, forming dynamic topological chromatin domains. Interfering with specific binding sites, by changing some red binding sites into blue, promotes passive looping of extended chromatin fibres, phenomena observed in vivo.

with long-range regulation, and how they might be established through different chromatin epigenetic states. With Prof. Mario Nicodemi (Univ. Naples, Italy), we have shown that available data on chromatin contacts, both originated by 3C approaches and fluorescence in situ hybridization (FISH), can be explained with the Strings & Binders Switch (SBS) model (Barbieri et al. 2012, 2013). Currently, we are using the SBS model to explore the 3D topology of the HoxB locus in mouse ES cells by simulating chromatin occupancy maps of epigenetic features as interaction sites. The HoxB polymer is mixed in silico with binders with affinity to the binding sites defined according to the epigenetic states across the locus. Binding induces polymer folding based on universal thermodynamics principles. Our current work combining polymer modeling using the SBS model with single-cell imaging by cryo-FISH shows that the 3D chromatin folding of the HoxB locus in ES cells can be explained by homotypic associations between Active and Polycomb-target genes. We will extend our analyses to understand changes in chromatin topology during cell commitment by modeling chromatin folding in neuronal precursor cells and terminally differentiated neurons. To this end, we are analyzing HiC datasets produced in collaboration with Dr. Josée Dostie (McGill University, Montreal).

To investigate the relevance of nuclear positioning for gene expression outcomes, we have also explored changes in gene positioning that occur in Huntington's disease and the nuclear positioning of the stochastically-expressed Ifnb gene in response to viral infection.

Selected Publications

Ferrai C, Xie SQ, Luraghi R, Munari D, Ramirez F, Branco MR, Pombo A, Crippa MP (2010) Poised transcription factories prime silent uPA genes prior to activation. PLoS Biology 8, e1000270.

Möller A, Xie SQ, Hosp F, Lang B, Phatnani HP, James S, Ramirez F, Collin GB, Naggert JK, Babu MM, Greenleaf AL, Selbach M, Pombo A (2012) Proteomic analysis of mitotic RNA polymerase II complexes reveals novel interactors and association with proteins dysfunctional in disease. Molecular Cellular Proteomics 11(6):M111.011767.

Brookes E, de Santiago I, Hebenstreit D, Morris KJ, Carroll T, Xie SQ, Stock JK, Heidemann M, Eick D, Nozaki N, Kimura H, Ragoussis J, Teichmann SA, Pombo A (2012) Polycomb associates genome-wide with a specific RNA polymerase II variant, and regulates metabolic genes in ES cells. Cell Stem Cell 10, 157-170.

Barbieri M, Chotalia M, Fraser J, Lavitas LM, Dostie J, Pombo A, Nicodemi M (2012) Complexity of chromatin folding is captured by the Strings & Binders Switch model. Proc. Natl. Acad. Sci. USA 109, 16173-16178.

Barbieri M, Fraser J, Lavitas LM, Chotalia M, Dostie J, Pombo A, Nicodemi M (2013) A polymer model explains the complexity of large-scale chromatin folding. Nucleus 4, 267-273.

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Photo: David Ausserhofer/MDC

Nikolaus Rajewsky

Systems Biology of Gene Regulatory Elements

My lab uses experimental (molecular biology, biochemistry) together with computational methods (bioinformatics, computational biology, etc) to dissect, systems-wide, function and evolution of gene regulation in metazoans. One major focus is to understand more about post transcriptional gene regulation exerted by small RNAs, in particular microRNAs. We are developing predictive models for targets of microRNAs. We also investigate general mechanisms of gene regulation by microRNAs and RNA binding proteins in cell lines and in vivo. For example, we are studying function and mechanisms of post-transcriptional gene regulation during early development in C. elegans. Furthermore, we have established planaria as a model system in our lab. These freshwater flatworms are famous for their almost unlimited ability to regenerate any tissue via pluripotent, adult stem cells. We investigate molecular mechanisms of pluripotency and the role of post-transcriptional gene regulation in planarian stem cell biology and regeneration.

Introduction

A major lesson from recent genomics is that metazoans share to a large degree the same repertoire of protein-encoding genes. It is thought that differences between cells within a species, between species, or between healthy and diseased animals are in many cases due to differences in when, where and how genes are turned on or off. Gene regulatory information is to a large degree hardwired into the non-coding parts of the genome. Our lab focuses on decoding transcriptional regulation (identification and characterization of targets of transcription factors in non-coding DNA) and post-transcriptional control mediated by RNA binding proteins and small, non-coding RNAs, in particular microRNAs. microRNAs are a recently discovered large class of regulatory genes, present in virtually all metazoans. They have been shown to bind to specific cis-regulatory sites in 3' untranslated regions (3' UTRs) of protein-encoding mRNAs and, by unknown mechanisms, to repress protein production of their target mRNAs. Our understanding of the biological function of animal microRNAs is just beginning to emerge, but it is clear that microRNAs are regulating or involved in a large variety of biological processes and human diseases, such as developmental timing, differentiation, signalling, homeostasis of key metabolic gene products such as cholesterol, cardiovascular diseases and cancer. Overall, however, it is clear that miRNAs are only a small part of the entire post transcriptional gene regulation apparatus used by cells, and we are beginning to systematically explore the largely unknown function of RNA binding proteins.

It is clear that a better understanding of gene regulation and in particular of the just emerging universe of non-coding RNAs can only come by integrating various data sources (comparative sequence analysis, mRNA expression data, protein-protein interactions, mutant phenotypes from RNAi screens, polymorphism data, experimentally defined gene regulatory networks, ChIP-chip data, etc) since each data source alone is only a partial description of how cells function. For example, to understand microRNA func-

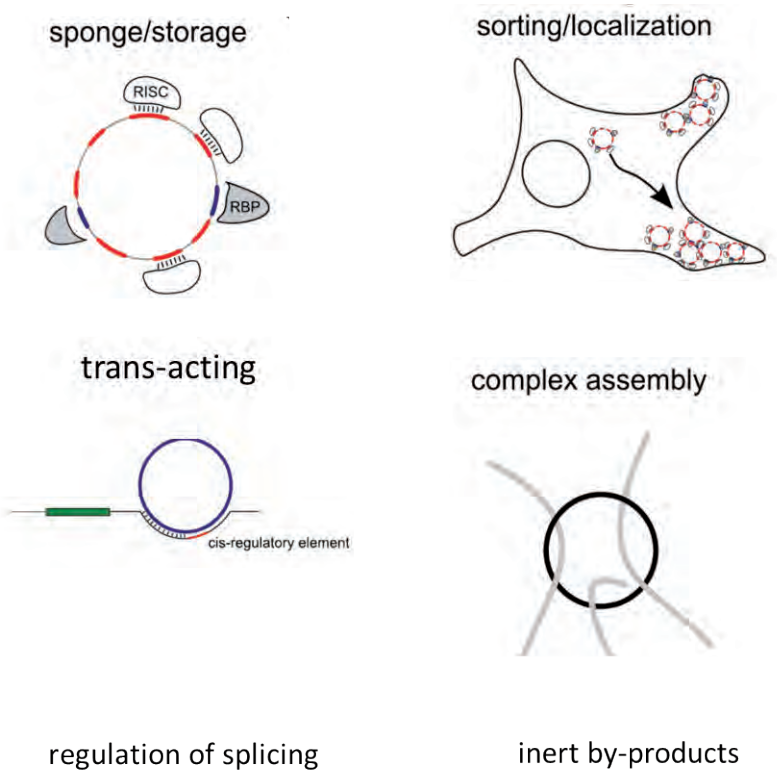


Figure 1: *Possible functions of circRNAs: (a) binding miRNAs or RNA binding proteins (RBPs) to change their local free concentration (b) transport of miRNAs/RBPs inside the cell (c) acting in trans (d) involved in assembly of other molecules (e) involved in regulating splicing (f) it is also a possibility that most circRNAs, after production, have no function.*

tion, we not only need to identify their targets but also to decode how microRNAs are transcriptionally regulated. A major focus of the lab is therefore in developing methods that integrate different data sources and methods to produce global and yet specific predictions about how, when, and where genes are regulated. This will ultimately lead to the identification and functional description of gene regulatory networks. We will continue to test, develop and "translate" these methods and their predictions using specific biological systems, such as metabolism in mammals, regeneration in planarians, early embryogenesis in C. elegans.

Specifically, we have developed one of the first microRNA target finding algorithms and could later on show that microRNAs very likely regulate thousands of genes within vertebrates, flies, and nematodes (Krek et al., Nature Genetics 2005; Lall et al., Current Biology 2006; Gruen et al., PloS Computational Biology 2006). We have further helped to elucidate the function of microRNAs in pancreatic beta cells (insulin secretion), in liver (cholesterol level), and other systems. More recently, we have shown that microRNAs can leave cell type specific mRNA expression signatures on hundreds of genes (Sood et al., PNAS 2006), and that human genotyped SNP data can be used to explicitly demonstrate and quantify the contribution of microRNA targets to human fitness (Chen and Rajewsky, Nature Genetics 2007). We have further developed computational methods (miRDeep) to predict miRNAs from high throughput sequencing data (Friedlaender et al., Nature Biotechnology 2008). We have also pioneered approaches that allowed to experimentally assay, genome-wide, the impact of miRNAs on protein synthesis (Selbach et al., Nature 2008). A major ongoing effort is currently to use and develop several key high-throughput technologies for in vivo studies in C. elegans and planaria: high-throughput proteomics (SILAC), RNA sequencing, and new methods that allow the genome-wide identification of binding sites of RNA binding proteins. Part of this work involved collaborations with the Stoffel lab (Rockefeller University, Piano & Gunsalus labs (NYU), Chen and Selbach labs (MDC). Regarding miRNAs we are currently working on advanced methods to biochemically ligate miRNAs to target sites and thus to be able to obtain context-dependent, in-vivo maps of genome-wide miRNA:target interactions (Grosswendt et al. submitted). These maps must be interpreted by Systems Biology methods to understand or predict phenotypes.

Circular RNAs

We have recently shown that single-stranded circular RNAs (circRNAs) are a widespread class of animal RNAs with tissue and developmental stage specific expression (Memczak et al. Nature 2013). circRNAs are usually expressed from exons, are found in the cytoplasm, and are unusually stable. For a particular circRNA (CDR1as) we could show that this circRNA can function as a

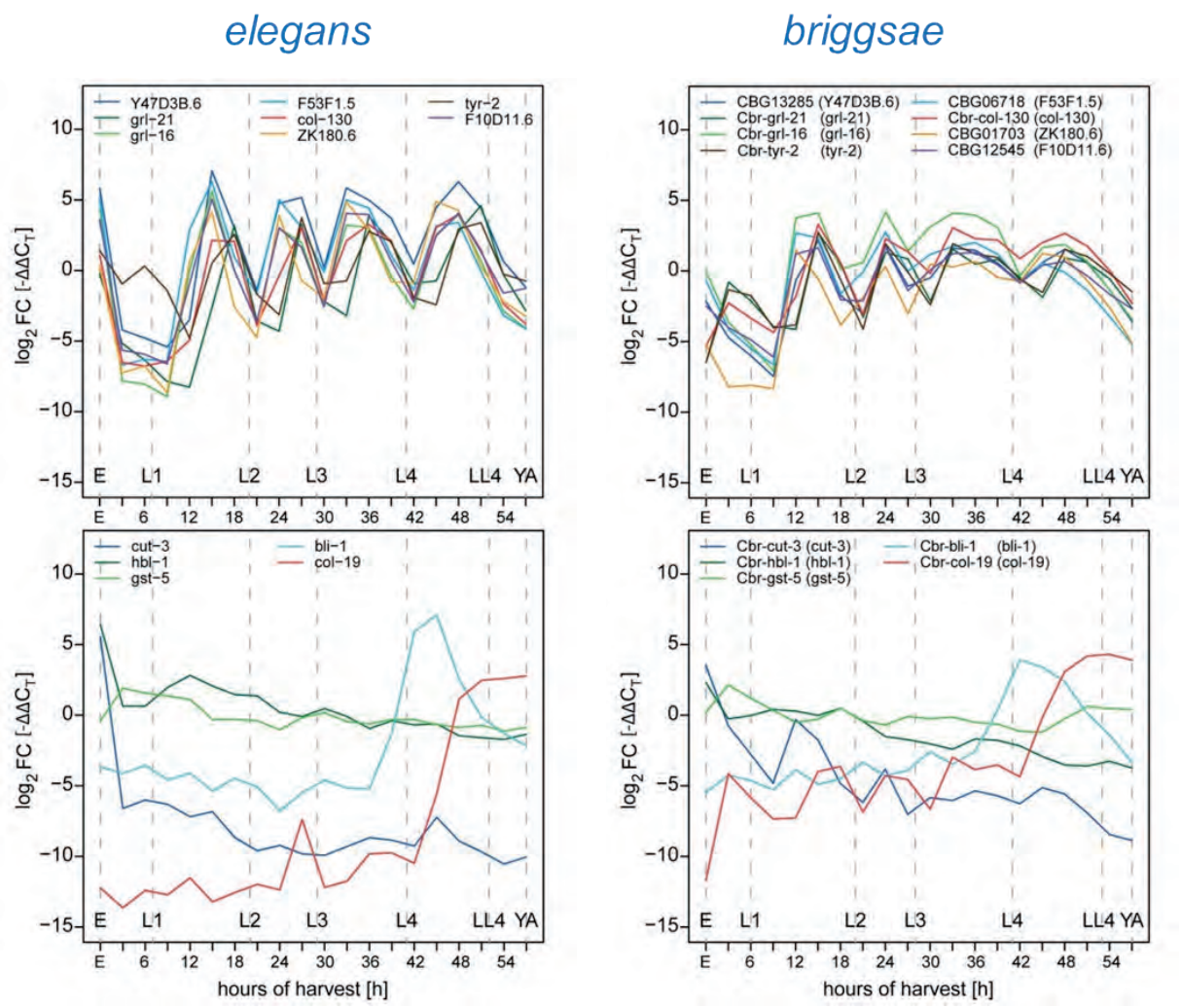


Figure 2: *By computational clustering of our data, we identified ~866 genes with cycling mRNA expression in C. elegans. The Figure shows qPCR data for randomly selected genes from this cluster (upper left panel) and negative controls (lower left panel). Orthologous genes were measured in C. briggsae (right upper and lower panels). Gruen et al., Cell Reports 2014.*

sponge to sequester miR-7, a neuronal specific miRNA. We are currently investigating possible functions of circRNAs and mechanisms of circRNA biosynthesis. Besides circRNAs potentially acting as sponges, we are studying a potential role of circRNA production in regulating splicing of mRNAs (Collaboration with the Kadener lab, Hebrew University, Jerusalem). However, it is also possible that circRNAs are largely inert by-products of splicing (Fig. 1). Even in this case, because of their unusual stability and differential expression, it will be extremely interesting to study circRNAs as possible biomarkers for processes.

Development in *C. elegans*

Although C. elegans is one of the most famous model systems for developmental biology, it has been impossible to use most high-throughput technologies to study differential gene expression and networks during very early embryogenesis (for example the oocyte to one-cell embryo transition upon fertilization). To overcome this problem, we have developed a novel method ("eFACS") that allows us to sort embryos at precise stages during embryogenesis via FACS sorting (Stoeckius, Maaskola et al., Nature Methods 2009). For example, we can now routinely obtain ~60,000 one-cell stage embryos (at a purity of >98%) in one FACS run, enough to apply virtually any high-throughput method of interest. We have used eFACS to assay the dynamics of small RNA expression during embryogenesis. We discovered a wealth of orchestrated, specific changes between and within virtually all classes of small RNAs. These findings open the door for many computational and functional follow up studies. For example, we have shown that sperm transmits RNA into the zygote, and that hundreds of paternal mRNAs/

small RNAs are present in the one-cell embryo (Stoeckius et al., submitted). We are also interested in understanding more global patterns of developmental gene expression. For example, in a recently published large-scale study we described the first systematic recording of RNA **and** protein levels during development. We recorded these levels in both elegans and briggsae species to be able to interpret our results. One surprising discovery was the existence of ~866 genes which cycle every ~8hrs in expression the animal. This cycling is conserved between species (Fig. 2) and thus likely functional. There is currently no explanation for this effect.

Stem cell biology

We used massive next generation sequencing to identify miRNAs and piRNAs in S. mediterranea. We also identified miRNAs that seem specifically linked to stem cell biology. A number of these miRNAs are conserved in humans (Friedlaender & Adamidi et al., PNAS 2009). We have further developed experimental and computational methods that allowed us to assemble the planarian transcriptome via next generation sequencing of mRNAs (Adamidi et al., Genome Research 2011). We have used FACS and subsequent RNA-seq and shotgun proteomics to obtain planarian stem cells and to define which genes are specifically expressed in them. Comparison to mammalian embryonic stem cells revealed that molecular mechanisms for pluripotency are deeply conserved throughout life and that planarian stem cells are indeed informative for mammalian stem cell biology (Oenal et al. EMBO J 2012). Part of this work involved collaborations with the Sanchez lab (Utah), Chen, Dieterich, and Kempa labs (MDC). Currently we are studying the role of alternative splicing in pluripotency.

Towards systematic decoding of the "post-transcriptional regulatory code"

We have started to use biochemical methods such as PAR-CLIP (Hafner et al., Cell 2010) to investigate mechanisms and function of post-transcriptional gene regulation. For example, we have studied the human RNA binding protein HuR. We identified >4000 functionally relevant targets of HuR and could show that HuR regulates mRNA processing and alternative splicing (Lebedeva et al., Molecular Cell 2011), previously unknown functions of HuR. Very interestingly, we also found that HuR directly and strongly controls the expression of one conserved human microRNA (miR-7). This finding illustrates the need for studying RNA binding proteins and microRNAs together. Ultimately we need to study regulatory relationships not in cell lines but in vivo. In a proof of principle experiment, we have shown that it is possible to biochemically identify targets of RBPs in C. elegans and at nucleotide resolution (Jungkamp et al., Molecular Cell 2011). This new method ("iPAR-CLIP") allows us to systematically identify the targets and to study the function of any RBP in C. elegans (Fig. 2). One immediate goal is to identify the targets of DICER and other central components of small RNA pathways in vivo (Rybak et al., submitted). Part of this work involved collaborations with the Landthaler, Selbach, and Kempa labs (MDC).

Selected Publications

Selbach M; Schwanhaeusser B; Thierfelder N; Fang Z; Khanin R; and Rajewsky N (2008). Widespread changes in protein synthesis induced by microRNAs. **Nature** 455, 58-63

Lebedeva S, Jens, M, Theil K, Schwanhaeusser B, Selbach M, Landthaler M, Rajewsky N (2011). Transcriptome-wide analysis of regulatory interactions of the RNA binding protein HuR. **Molecular Ce**ll 43, 340-352.

Jungkamp AC, Stoeckius M, Mecenas D, Mastrubuoni G, Kempa S, Rajewsky N (2011). In vivo and transcriptome wide identification of RNA binding protein target sites. **Molecular Cell** 44, 828-40.

Onal P, Grün D, Adamidi C, Rybak A, Solana J, Mastrobuoni G, Wang Y, Rahn HP, Chen W, Kempa S, Ziebold U, Rajewsky N (2012). Gene expression of pluripotency determinants is conserved between mammalian and planarian stem cells. **EMBO J** 31, 2755-69.

Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. **Nature** 495, 333-338.

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Photo: David Ausserhofer/MDC

Baris Tursun

Gene Regulation and Cell Fate Decision in *C. elegans*

Cell fate decisions are fundamental during development of multicellular organisms. While a few decisions can be reversed, most differentiated cells are protected against induced conversion of their identity [1]. The molecular mechanisms required for safeguarding different cell fates are not fully understood. We use C. elegans *as a genetic model organism to systematically study the molecular basis of cell fate maintenance. Specifically, we are interested in mechanisms counteracting experimental attempts to induce an ectopic identity in mature cells.* C. elegans *provides genetic tractability and the instant visualization of cell fate conversions* in vivo. *This allows unbiased large-scale interrogation of the genome for genes playing a role in safeguarding cell fates. Our findings could help to overcome limitations in induced* cellular transdifferentiation – *often referred to as* direct reprogramming – *and thereby support the development of future biomedical approaches.*

Forward genetics to identify inhibitors of inducing ectopic cell fates

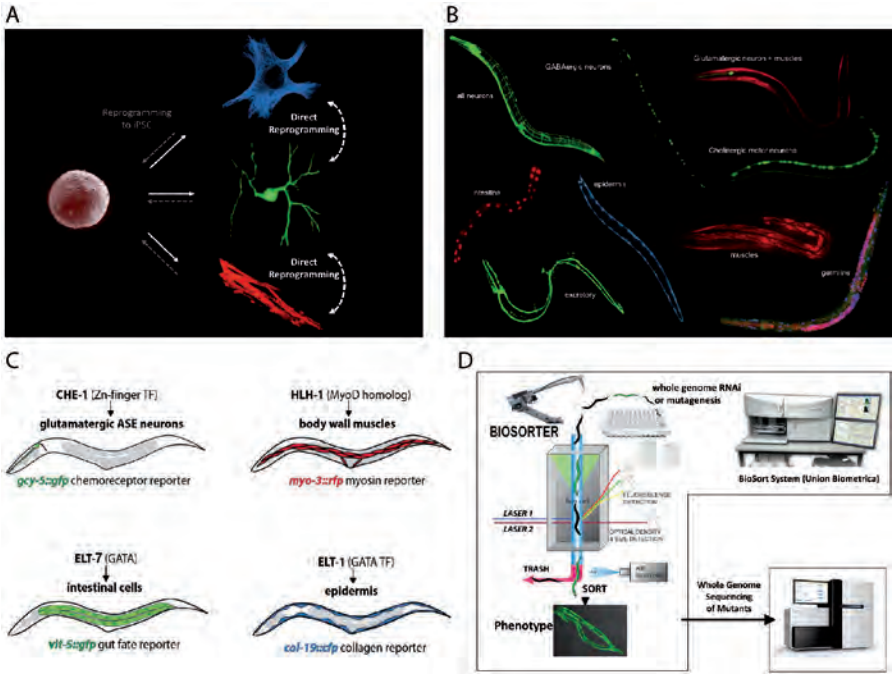
O. Kaplan, S. Bulut, A. Ofenbauer

In early *C. elegans* embryos, ectopic expression of specific transcription factors (TFs) can convert most cells into, e.g., neurons. In contrast, differentiated cells in adults cannot be reprogrammed as efficiently by mis-expressed TFs [2]. Partly, limitations in *direct reprogramming* can be bypassed by mis-expressing a cocktail of several different TFs [3]. But the actual issue, namely the molecular signature causing limitations in direct reprogramming, remains poorly understood. A straight strategy to identify factors counteracting induction of cell fates is to perform genetic screens. Forward genetics can be easily applied to *C. elegans* enabling us to systematically interrogate the entire worm genome for inhibitory factors. We are making use of transgenic worms that allow broad mis-expression of TFs *(cell fate master regulators),* which are sufficient to induce distinct tissue types. We use a glutamatergic neuron-inducing TF, and the myogenic TF MyoD (HLH-1 in worms), as well as GATA TFs that induce the intestinal cell fate [2]. Also, these worms express fluorescent reporters that label specific cell types thereby monitoring cell conversions *in vivo* (Fig.1C). Ongoing mutagenesis screenings delivered several mutants that allow the ectopic induction of neuronal fates in non-neuronal tissues. These are currently being characterized to assess the efficiency and depth of inducing neuronal characteristics in non-neuronal cells.

Reverse genetic screens to identify inhibitors of inducing ectopic fates

E. Kolundzic, M. Hajduskova, ML. Beato Del Rosal, M. Kazmierczak

Complementary to our forward genetic screens we are performing whole-genome RNAi screens (knock-down of 20K genes). Since evidence from previous studies suggest that repressive chromatin signatures counteract the induction of ectopic cell fates, we have created an RNAi sub-library that targets all *C. elegans* chromatin factors. The sub-library allows expedite examination of reprogramming regulation by epigenetic factors using different conditions. It enables us to systematically apply a variety of parameters in order to genetically enhance partial cell fate conversions. Manual screens can be laborious and slow but systematic and combinatorial genetic interrogations to reveal multigenic phenotypes require high-throughput approaches. We are using



A: We are studying limitations of directly converting cellular identities in vivo. *B: The genetic model organism* C. elegans *provides genetic tractability and instant* in vivo *visualization of cell types. C: Transgenic worms allow broad mis-expression of* master regulatory *transcription factors (TFs) that induce glutamatergic neurons (CHE-1), the muscle fate (HLH-1/MyoD), and GATA TFs that induce the intestinal or epidermal cell fates. D: The BioSorter® (Union Biometrica) allows high-throughput scanning of 20 worms/sec and simultaneous detection of three fluorescent channels. An auto-sampling robot allows using multi-well plates for automated RNAi screening. Mutants derived from forward genetic screens are subject to whole genome sequencing to identify mutated genes.*

the BioSorter® which screens 20 worms per second thereby reducing the time for a whole genome RNAi screen from 250 (manual) to 40 days (Fig.1D). Moreover, it provides simultaneous detection of three fluorescent channels with high sensitivity. Ongoing manual screens yielded a number of factors, whose depletion allow ectopic induction of neuronal reporters in epidermal, intestinal or germ cells. Such RNAi based phenotypes will be confirmed using mutants for the respective genes (the *Caenorhabditis Genetics Center* provides mutants for more than 90% of all worm genes).

The histone chaperone RBBP4/7 is required for muscle maintenance

S. Seelk, M. Hajdusjova

The histone chaperone LIN-53 (human RBBP4/7) was previously shown to protect germ cells from direct conversion into neurons or muscles [4,5]. Genetic data indicate an interaction of Notch signaling with LIN-53 regulation for safeguarding germ cells. We are currently characterizing the nature of this intriguing interaction.

LIN-53 is expressed in all tissues but its function in different cell types is not clear. Tissue-specific protein biochemistry is being applied to characterize LIN-53 in detail. Initial molecular and cell biological analysis revealed mis-regulation of critical TFs required for muscle differentiation and maintenance in *lin-53* mutants. Hence, mutants show strong muscle and motility defects and, additionally, a short lifespan.

We expect that our efforts will yield a number of different factors and mechanisms to better understand differentiation, maintenance and safe-guarding of cell fates. The *C. elegans* genome is ~60% homologous to those of mammals such that our large-scale systematic approaches might lead to identification of conserved mechanisms.

Reference:
[1] Graf T. *Cell Stem Cell* 2011
[2] Tursun B. *Curr Opin Genet Dev* 2012
[3] Ladewig J, Koch P, Brüstle O. *Nat Rev Mol Cell Biol* 2013
[4] Tursun B, Patel T, Kratsios P, Hobert O. *Science* 2011
[5] Patel T, Tursun B, Rahe DP, Hobert O. *Cell Reports* 2012.

Selected Publications

Cochella L*, Tursun B*, Hsieh YW, Galindo S, Johnston RJ, Chuang CF, Hobert O#.
Two distinct types of neuronal asymmetries are controlled by the Caenorhabditis elegans zinc finger transcription factor die-1.
Genes Dev. 2014 Jan 1;28(1):34-43.

Patel T*, Tursun B*, Rahe DP, Hobert O#.
Removal of Polycomb repressive complex 2 makes C. elegans germ cells susceptible to direct conversion into specific somatic cell types.
Cell Rep. 2012 Nov 29;2(5):1178-86.

Tursun B#.
Cellular reprogramming processes in Drosophila and C. elegans.
Curr Opin Genet Dev. 2012 Oct;22(5):475-84.

Tursun B*#, Patel T, Kratsios P, Hobert O#.
Direct conversion of C. elegans germ cells into specific neuron types.
Science. 2011 Jan 21;331(6015):304-8.

Tursun B*#, Cochella L*, Carrera I, Hobert O#.
A toolkit and robust pipeline for the generation of fosmid-based reporter genes in C. elegans.
PLoS One. 2009;4(3):e4625.

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Photo: David Ausserhofer/MDC

Robert P. Zinzen

Systems Biology of Neural Tissue Differentiation

How cells acquire and maintain their identity remains a fundamental question in biology. We aim to gain a detailed understanding of the molecular mechanisms and paths that undifferentiated embryonic cells take to organize into distinct cell types and tissues. With the advent of modern '-omics' approaches, it is becoming increasingly possible to investigate this problem systematically and on a genome-wide scale. No longer limited to asking how individual genes are regulated across development, we can now start asking how the genome at large is regulated to coordinate development. We approach this problem in the context of early embryonic development in the fruit fly Drosophila melanogaster (D.mel.), because of its ideal genetic tractability and experimental accessibility. We are primarily interested in the nervous system (NS), which derives from a large population of cells specified very early. This pluripotent primordium quickly subdivides into columnar domains that give rise to specific neuroblasts. Our aim is to understand the molecular mechanisms underlying NS primordium specification, NS subdivision, as well as the precisely regulated spatio-temporal delamination of differentiating neuroblasts.

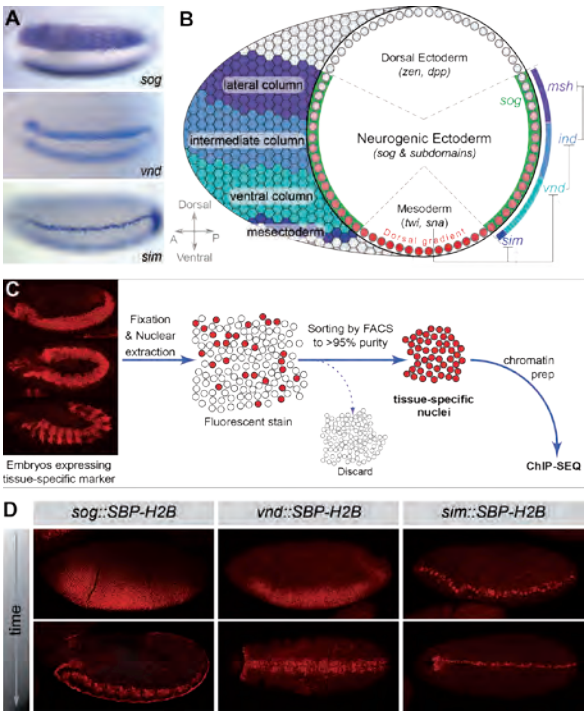
Gene- and *cis*-regulatory networks for NS development

L. Dämpfling, A. Klawiter, P. Wahle

Coordinated specification and differentiation in development is driven to a large degree via tight spatio-temporal regulation of gene expression. Whether a cell will express a particular gene is directed by how that gene's *cis*-regulatory elements (CRMs) interpret the cellular environment, e.g. which transcription factors (TFs) can be recruited to enhancers. Several TFs crucial for NS patterning are known and we are utilizing chromatin immunoprecipitation (ChIP) followed by massively parallel sequencing (Seq) to identify the genome-wide binding profiles of these TFs. We aim to identify the target genes of neurogenic TFs, the CRMs through which they act, and how TFs act combinatorially. This is a crucial step for understanding a TF's global regulatory potential, how TF combinations coordinate regulatory networks, and how these networks change across development.

A caveat of ChIP-Seq studies is that binding data cannot definitively identify target genes or predict transcriptional responses. To assess the effect that individual TFs have on global transcription in vivo, we are utilizing the genetic tractability of *D. mel.* by fluorescently marking embryos depending on the mutations they carry. We will use a large-particle sorter to isolate pools of staged, homozygous-mutant embryos to analyze the effect of key neurogenic TFs on the developmental transcriptomes of specific NS compartments.

While some of the key TFs we are interested in are expressed in defined subsets of the NS, others are employed more broadly. For example, opposing morphogen gradients signal via effector molecules to shape transcription across the NS in a concentration-dependent manner. To understand how the morphogen gradients are interpreted at the DNA level across the developing NS *in vivo*, we are analyzing the genome-wide binding profiles of effector TFs in specific NS components isolated from developing embryos – such tissue specific analyses have only recently become possible due to methodological advances (e.g. BiTS-ChIP, see below).



Patterning and dissection of the early Drosophila nervous system.
A) Early signs of NS specification and subdivision in the cellularizing embryo: sog mRNA expression demarks the extent of the neurogenic ectoderm (NE) from which the NS derives, vnd demarks the ventral NE that gives rise to the ventral column of the NS, sim marks the ventral-most row of cells that will form the ventral midline upon gastrulation. Embryos are shown anterior left and dorsal up.
B) Schematic representation of early NE subdivision; marker genes for the individual columns and their known genetic interactions are indicated.
C) Workflow of the BiTS procedure for the isolation of tissue specific nuclei from staged, fixed embryos.
D) Three transgenic lines for BiTS-ChIP-Seq, which direct expression of an SBP-tagged nuclear protein (visualized in red) in different parts of the developing NS.

Tissue specific chromatin dynamics

A. Glahs, J. Rossius

A more encompassing view of genome regulation will be obtained by investigating chromatin state over the course of NS development. We and others have shown that enrichment of specific histone modification combinations is not only predictive of genome features (enhancers, promoters, etc.), but also of their regulatory state (active, repressed, etc.). However, since chromatin features are employed differentially across the embryo, tissue specific signatures can only be identified if chromatin is first isolated from the cell populations of interest. BiTS (Batch isolation of Tissue Specific chromatin) allows just that by leveraging tissue specific transgenic markers to isolate fixed nuclei of defined cell populations by FACS, which are then used for ChIP-Seq. We have been establishing transgenic D.mel. lines that allow for the identification of tissue specific chromatin signatures in the NS, its columnar components, as well as in non-NS tissues. This allows determination of chromatin state in NS components, identification of genomic regulatory features, and their activity dynamics across development. An important goal is the functional integration of TF binding and chromatin state data to understand how TFs shape their chromatin environment and how the chromatin environment regulates the interaction of TFs with the genome.

Network extension

S. Krüger

An central aim is to gain a mechanistic understanding of how individual CRMs translate their cellular environment into regulatory (in)activity; however, it is clear that our provisional networks of NS development are still very incomplete. To extend the NS networks, we are assembling transgenic constructs that allow for the tissue specific biochemical isolation of individual NS CRMs together with the proteins that bind to them *in vivo.* Using mass spectrometry to identify the bound proteins, we are particularly interested in how CRM activity is affected by both the temporal dynamics of CRM-protein interactions across development, as well as by the tissue specificity of CRM-protein complexes. With this method, we are not only approaching a mechanistic understanding of individual CRMs, but once identified, can probe novel interactors for their broader neurogenic roles.

Overall, our aim is to establish a predictive model of NS development: we want to understand the regulatory interactions driving tissue development to a degree that allows modeling the global effects of systematic perturbations. Additionally, we will assay the general applicability of regulatory principles learned in the context of *D. mel.* NS development to other tissues and organisms.

Selected Publications

Bonn S, Zinzen RP, Perez-Gonzalez A, Riddell A, Gavin AC, Furlong EE (2012). Cell type-specific chromatin immunoprecipitation from multicellular complex samples using BiTS-ChIP. Nature Protocols. 7 (5): 978-994.

Zinzen RP, Bonn S, Girardot C, Gustafson EH, Perez-Gonzalez A, Delhomme N, Ghavi-Helm Y, Wilczynski B, Riddell A, Furlong EE (2012). Tissue-specific analysis of chromatin state identifies temporal signatures of enhancer activity during embryonic development. Nature Genetics. 44 (2): 148-156.

Zinzen RP, Girardot C, Gagneur J, Braun M, Furlong EE (2009). Combinatorial binding predicts spatio-temporal cis-regulatory activity. Nature. 462 (7269): 65-70.

Zinzen RP, Cande J, Ronshaugen M, Papatsenko D, Levine M (2006). Evolution of the ventral midline in insect embryos. Developmental Cell. 11 (6): 895-902.

Zinzen RP, Senger K, Levine M, Papatsenko D (2006). Computational models for neurogenic gene expression in the Drosophila embryo. Current Biology. 16 (13): 1358-1365.

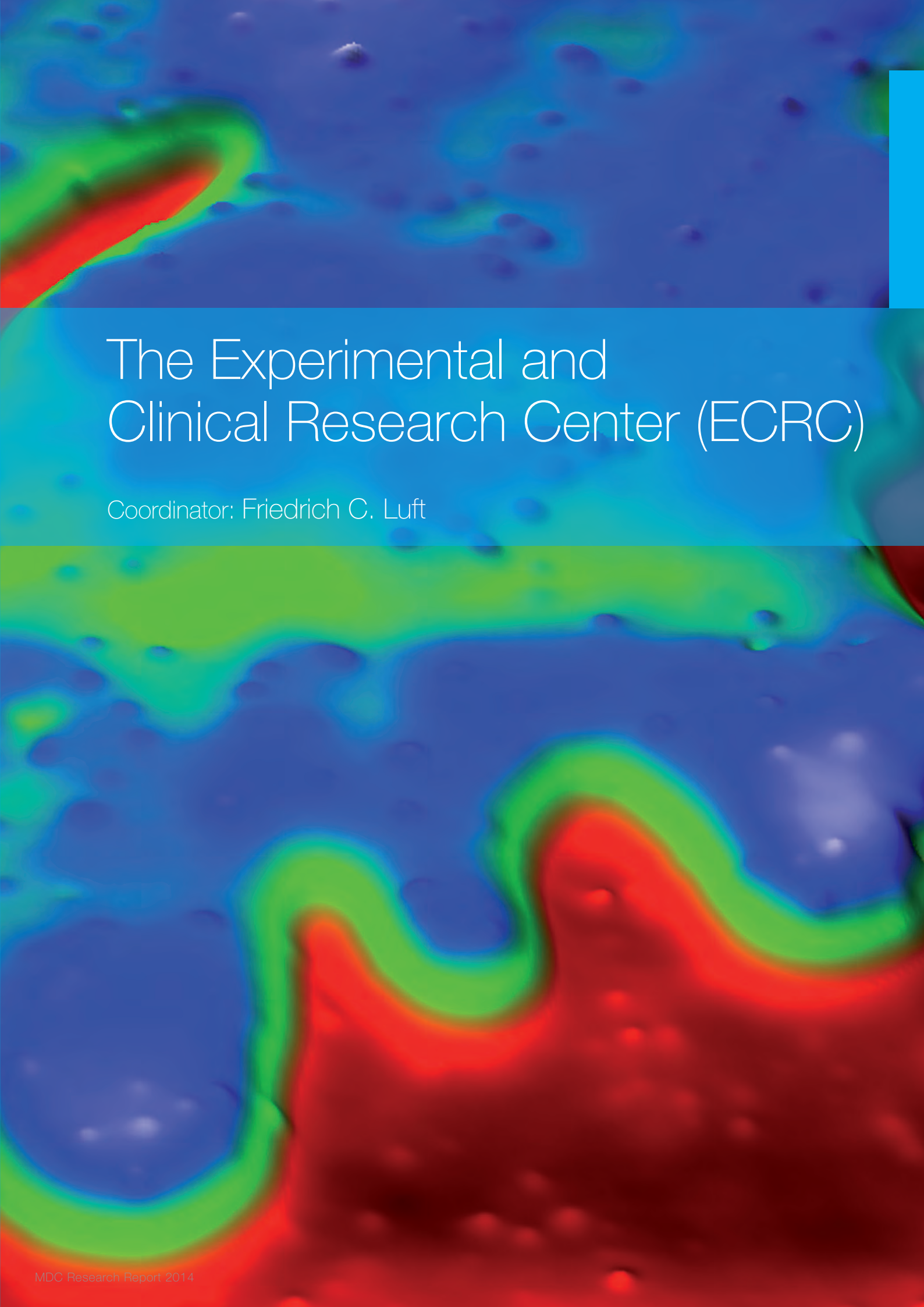
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The Experimental and Clinical Research Center (ECRC)

Coordinator: Friedrich C. Luft

The Experimental and Clinical Research Center (ECRC)

Friedrich C. Luft

The ECRC is a joint project of the MDC and the Charité. The mission of the ECRC is to pursue basic science directly relevant to clinical medicine. The notion of bringing novel ideas to patient care more quickly is termed "translational" research. The concept is not new. For instance insulin was discovered in 1921 and given to a diabetic patient in 1922. The Nobel Prize was awarded for this work in 1923. The ECRC is there to keep that goal alive, but we are not naïve. The 2005 international review panel founding the ECRC recognized that the ECRC needed dedicated well-trained clinicians heading research groups. These clinicians deserved a state-of-the-art infrastructure. Equally important, the clinicians required world-class basic scientists, who are committed to working on clinical problems with clinicians that are generally less knowledgeable in the science. These marriages are seldom made in heaven. Who remembers Bertram Collip, the biochemist who purified insulin so that the compound could actually be given to a human? Without him, no treatment, no prize, and more importantly no cure.

The ECRC was designed as an interface between MDC scientists and Charité clinician/scientists. We are located in a previous hospital building named after Robert Rössle, a Charité pathologist in the Virchow line of succession. ECRC groups acquire laboratory space and infrastructure through competition. The space is provided for five years and the contract can be renewed, again on a competitive basis. Scientific productivity is measured in publications and grant support. Not all, but most ECRC groups are led by clinicians who also have patient-care responsibility at one of the Charité campuses and/or in our outpatient facility. Two of our group leaders are clinicians at the HELIOS-Klinikum-Berlin, a large private hospital adjacent to the campus. The ECRC provides salary support for clinician-scientists. In this way, we address the problem of "protected time", which is not generally available for persons on a clinical salary line.

Recruitment, mentoring, and interactions are our main tools at the ECRC. To entice clinicians into a scientific career, we initiated a research fellowship for clinicians at the MDC years ago. This fellowship for two years with extension enables clinicians to accrue scientific expertise and possibly an additional degree. The program has been very popular and has been adapted institution-wide by the Charité. Subsequent career development is a problem; however, two possibilities are attachment to an existing ECRC group or establishing a new independent group. Another challenge is bringing MDC scientists and clinician/scientists together. We have developed a partnership granting system to that end to foster such collaborations.



Photo: David Ausserhofer/MDC

***Fig. 1. P**hilipp Du Bois and Jens Fielitz explain their project to Angela Merkel, while Friedrich Luft looks on.*

The ECRC has a facility that exists nowhere else in Berlin, although that state-of-affairs may change in the future. We have a designated clinical research unit (CRU) to study humans, outside of the health-care system and solely for the purposes of science. This unit is operative since 2000 and has been extremely productive in fostering investigator-initiated mechanistic research. Much very valuable clinical research can be performed in a patient-care setting. Most clinical research viewed by the public fits this venue. For instance, comparing tablet A with tablet B, or new cancer treatment X compared to old cancer treatment Y, or coated stent Q versus bare-metal stent R needs no special unit because extra resources to test these questions are minimal. More difficult are studies involving "new ideas". Just suppose we develop a new stem-cell therapy for a genetic or acquired human disease. The commercial application is not obvious and the health-care system is not enamored with the idea of supporting this endeavor. That is where the CRU steps in.

The uniqueness of the ECRC is the mission. We are at the source of medically related basic science. The MDC is the best broad-based institute that Germany has to offer. Ideas outside the MDC could be channeled via that source. Our medical school has the entire city of Berlin at its disposal. Thus, patient recruitment (from 4 million inhabitants) is possible as is nation-wide recruitment. We view "translational research" as our job. We admit that we cannot guarantee beating the diabetes team of Banting, Best, McLeod, and Collip. But this goal is a worthwhile aim.

Research on human subjects in the Clinical Research Unit (CRU)

Patient/proband-oriented research is the final step in bringing medical innovations to humans or in revealing physiological or pathological mechanisms in man. This bench-to-bedside and bedside-to-bench process is today conducted under the trendy catchword "translational" research. Our CRU is modeled after the US general clinical research centers and has functioned well since 2000. We have succeeded in making the CRU independent of any patient-care endeavors that concern third-party payers (cross financing). We have recruited physicians, nurses, scientists, technical experts and students.

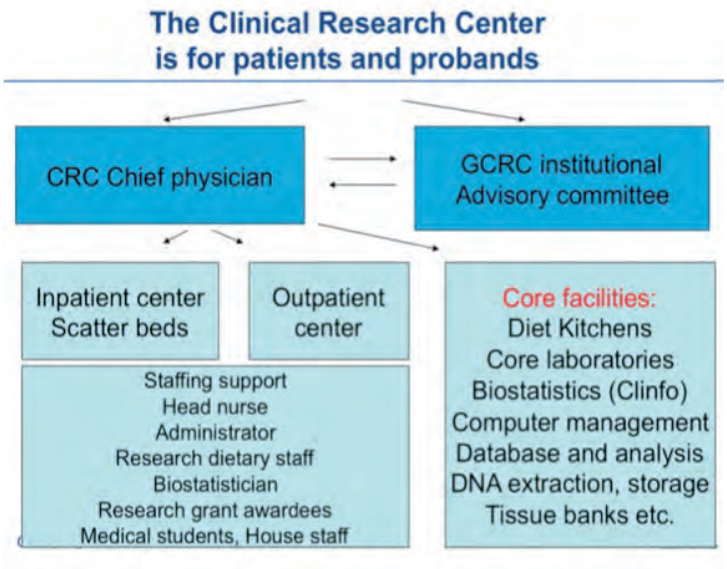


Fig. 2. *Our CRU organizational concept is adapted from the general clinical research center idea formulated by NIH. We supply infrastructure and various services for any investigator approved by the Institutional Advisory Committee.*

Our CRU is located in a former hospital building and relies on excellent facilities included the former new bone-marrow transplant unit. We are well equipped for cardiovascular and metabolic studies with resources not available elsewhere, including a normobaric hypoxia chamber, a total-body metabolic chamber, and a large gamut of techniques including microdialysis, spiro-ergometry, muscle nerve recordings, lower-body negative pressure, insulin and glucose-clamp studies, and indirect calorimetry. We also conduct detailed body composition measurements. We adapt our capabilities to the investigator's needs. Any clinical investigator at the Charité is eligible to use our facility and its infrastructure after applying to and receiving approval by our Institutional Advisory Committee.

We have not focused on inpatient studies but could do so if required. As a result, for

Fig. 3. *Michael Boschmann introducing notable guests to the CRU metabolic chamber.*



pharmacological investigations we currently are not doing phase 1 studies but conduct phase 2 and phase 3 clinical trials, although our focus has been primarily on physician-investigator initiated mechanistic research. We have cooperated actively with the MDC magnetic resonance imaging center, particularly with Jeanette Schulz-Menger. Our mission is also directed at supporting our outpatient clinics. In the outpatient clinics, we have excellent ultrasound capabilities including state-of-the-art echocardiography and a laboratory aimed at biobanking samples appropriately.

Human Data Management

For data management of all human data, we use REDCap, a clinical data management system (also called electronic case report form) developed at Vanderbilt University in 2004. The REDCap Consortium is composed of 873 active institutional partners in 71 countries and is currently used in more than 89,000 projects with over 116,000 users. Thus, REDCap is one of the most frequently used clinical data management systems worldwide. Our group has joined the REDCap consortium. The entire program can be inspected at: www.project-redcap.org. We will now manage all our outpatient contacts with an electronic medical records system that we will install in 2014. The Regenstrief Institute is an internationally respected informatics and healthcare research organization, recognized for its role in improving quality of care, increasing efficiency of healthcare delivery, preventing medical errors and enhancing patient safety. The Regenstrief Institute, Inc., an internationally recognized informatics and healthcare research organization, is dedicated to the improvement of health through research that enhances the quality and cost-effectiveness of health care, by conducting health-services research (HSR). HSR works to improve healthcare delivery and health outcomes of patients through research on patient safety, quality of care, patient-provider communication strategies, applied health information technologies, system redesign, and medical education research. This healthcare information technology can be inspected at: *www.regenstrief.org.*

Recent CRU Publications

Haufe S, Haas V, Utz W, Birkenfeld AL, Jeran S, Böhnke J, Mähler A, Luft FC, Schulz-Menger J, Boschmann M, Jordan J, Engeli S. Long-lasting improvements in liver fat and metabolism despite body weight regain after dietary weight loss. Diabetes Care. 2013;36:3786-92.

Haufe S, Utz W, Engeli S, Kast P, Böhnke J, Pofahl M, Traber J, Haas V, Hermsdorf M, Mähler A, Busjahn A, Wiesner S, Otto C, Mehling H, Luft FC, Boschmann M, Schulz-Menger J, Jordan J. Left ventricular mass and function with reduced-fat or reduced-carbohydrate hypocaloric diets in overweight and obese subjects. Hypertension. 2012;59:70-5.

Boschmann M, Nussberger J, Engeli S, Danser AH, Yeh CM, Prescott MF, Dahlke M, Jordan J. Aliskiren penetrates adipose and skeletal muscle tissue and reduces renin-angiotensin system activity in obese hypertensive patients. J Hypertens. 2012;30:561-6.

Singer E, Elger A, Elitok S, Kettritz R, Nickolas TL, Barasch J, Luft FC, Schmidt-Ott KM. Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. Kidney Int. 2011;80:405-14.

Lechner SG, Markworth S, Poole K, Smith ES, Lapatsina L, Frahm S, May M, Pischke S, Suzuki M, Ibañez-Tallon I, Luft FC, Jordan J, Lewin GR. The molecular and cellular identity of peripheral osmoreceptors. Neuron. 2011;69:332-44.

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Outpatient clinics

Psychiatry (Memory and cognition)
Psychiatry (Post-stroke depression)
Psychiatry (Fragile X syndrome)
Neurology (Skeletal muscle disorders)
Neurology (Inflammatory CNS disorders)
Cardiology (Cardiomyopathy)
Nephrology (Diabetes registry)
Metabolism (Diabetes and lipids)
Pediatrics (Pulmonary/allergic disease)



Photo: Peter Himsel/BBB GmbH

Simone Spuler

Muscle Research Unit and Outpatient Clinic for Muscle Disorders of the Charité

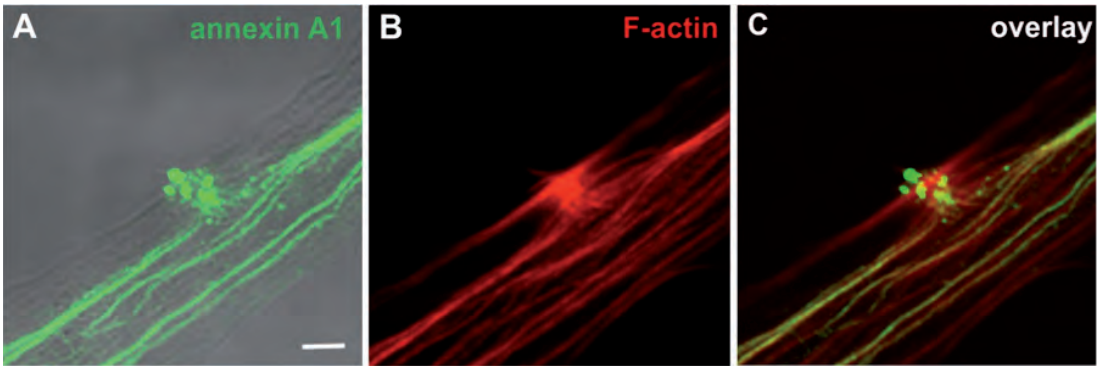
Simone Spuler directs the skeletal muscle disease program project (DFG_KFO192) for the entire Charité. Much of the research activities are located at the ECRC. The outpatient clinic at the ECRC cares for >1000 patients with genetic and acquired skeletal muscle diseases. In 2010, KFO192 was complemented by the international research-training grant for myology, "MyoGrad" (GK1631), recently recommended for renewal 2014 - 2019. This worldwide unique training grant crosses national boundaries and unites the Université Pierre et Marie Curie in Paris, Université St. Quentin en Yvelines in Versailles (French Speakers, Thomas Voit and Helge Amthor), with the Berlin located institutions: Free University, Charité Medical Faculty and Max Delbrück Center for Molecular Medicine (German Speaker, Simone Spuler). A binational degree (cotutelle de thèse) can be achieved by graduation within MyoGrad.
The scientific focus of Simone Spuler's group is the pathophysiology and metabolic consequences of muscular dystrophies caused by genes encoding sarcolemmal proteins (dysferlin, caveolin 3) and related acquired disorders (statin-myopathy, critical illness myopathy). Gene repair is a cooperative interest in MyoGrad subproject P6, headed by Simone Spuler and Luis Garcia.

Dysferlinopathies

Dysferlin, a large protein located at the sarcolemma, is involved in membrane repair after microinjury, a physiological consequence of normal muscle activity. Mutations in dysferlin lead to muscular dystrophy. Sarcolemmal repair is a complex multicomponent process. We demonstrated that the membrane repair process always involves the accumulation of F-actin and identified the dynamin-related ATPase EHD2 as a new component of the membrane repair complex (Marg et al., 2012). In future studies Andreas Marg and Tobias Timmel will dissect the repair process further.

Dysferlinopathies not only lead to faulty membrane repair but appear to lead to specific metabolic alterations in skeletal muscle. We found accumulation of lipids in dysferlin-deficient type 2 fibers (Grounds MD et al., Am J Path 2014, in press). Stefan Kempa's group at the BIMSB and our group are cooperatively investigating the mechanism. Verena Schöwel demonstrated that missense-mutated dysferlin can be rescued to function in vitro if the mutated protein is reallocated to the sarcolemma (Schoewel et al., 2012). She generated a new mouse model harboring a missense mutant in Dysf and will continue her studies in vivo.

Susanne Philippi, Jakub Malcher and Helena Escobar repair dysferlin at pre-mRNA or DNA level with the overall aim to find a cure for dysferlin-deficient muscular dystrophy. These are close cooperations with Luis Garcia, Université St. Quentin en Yvelines and Zsuzsanna Iszvák (MDC).



Annexin A1- and F-actin accumulation at the wounding area after laser irradiation
(A) Annexin A1 immunostaining of a human myotube shows an enrichment of the protein after laser wounding. (B) Phalloidin-atto 565 staining demonstrates the accumulation of F-actin at the same membrane area. (C) Overlay of A and B.

Satellite cells

Two separate repair mechanisms ensure the survival of muscle fibers: Membrane repair after minute injury to the sarcolemma as outlined above and new formation of muscle via activating the stem cells proper of skeletal muscle, the satellite cells. Muscle satellite cells have the full potential to proliferate, differentiate, and conduct repair. We are exploring the potential of human satellite cells derived from adult donors to regenerate muscle. These investigations also aim towards a therapy of muscular dystrophies.

Acquired myopathies: Statin induced myopathy and metabolic aspects of critical illness myopathy

Inhibitors of 3-hydroxy-3-methylglutaryl-coenzym-A-reductase (statins) are prescribed to millions of patients of whom 10% develop muscle related side effects. We are exploring genetic and structural aspects of this common myopathy. Critical illness myopathy occurs as a severe side effect of intensive care unit treatment. In a large translational and interdisciplinary approach that involved several ECRC groups we have shown that the failure to activate AMPK and to translocate GLUT4 to the sarcolemma contributes significantly to muscular atrophy.

Primary funding sources
Deutsche Forschungsgemeinschaft (KFO192 and MyoGrad IGK1631).

Selected Publications

Marg A, Schoewel V, Timmel T, Schulze A, Shah C, Daumke O, Spuler S. Sarcolemmal repair is a slow process and includes EHD2. Traffic. 2012;13:1286-94.

Schoewel V, Marg A, Kunz S, Overkamp T, Siegert Carrazedo R, Zacharias U, Daniel PT, Spuler S. Dysferlin-peptides reallocate mutated dysferlin thereby restoring function. PloS One. 2012; 7: e49603.

Weber-Carstens C*, Schneider J*, Wollersheim T, Assmann A, Bierbrauer J, Marg A, Al Hasani H, Chadt A, Wenzel K, Koch S, Fielitz J, Kleber C, Faust K, Mai K, Spies CD, Luft FC, Spranger J, Spuler S. Critical illness myopathy and GLUT4 – significance of insulin and muscle contraction. Am J Resp Crit Care Med 2013; 187: 387-96. *equal contribution

Timmel T, Schuelke M, Spuler S. Identifying dynamic membrane structures with atomic-force microscopy and confocal imaging. Microsc Microanal. 2014:1-7. [Epub ahead of print]

Grounds MD, Terrill J, Radley-Crabb H, Robertson T, Papadimitriou J, Spuler S, Shavlakadze T. Lipid accumulation in dysferlin-deficient muscles. Am J Pathol. 2014, [Epub ahead of print] PubMed PMID: 24685690.

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Photo: David Ausserhofer/MDC

Ralph Kettritz

Nephrology and Inflammatory Vascular Diseases

The group studies neutrophil functions in health and disease and molecular mechanism that control important neutrophil responses. In addition to protecting the host from infections, the neutrophil participates in autoimmunity, cardiovascular diseases, and cancer. Our main clinical interest deals with patients who suffer from systemic necrotizing vasculitis. This highly inflammatory vasculitis is caused by autoantibodies directed against neutrophil proteins. Patients develop either anti-neutrophil cytoplasmic autoantibodies (ANCA) to proteinase 3 (PR3) or antibodies directed at myeloperoxidase (MPO). ANCA bind to their target antigens on neutrophils and monocytes and these activated cells then interact with the endothelium leading to vascular damage. The disease is truly systemic in that each and every organ can be involved. Kidney and lung involvement is most frequent and associated with high mortality. Standard treatment consists of steroids, cytotoxic, anti-CD20 directed drugs, and plasma exchange. With these treatments patient and kidney survival has improved. However, treatment-related side effects are life-threatening. Improved understanding of disease mechanisms will result in better treatment.

Studying disease mechanisms in a murine ANCA disease model

We have developed an ANCA model for necrotizing crescentic glomerulonephritis (NCGN). In this model, anti-MPO antibodies activate neutrophils and monocytes and these cells cause the glomerular lesions. Several leukocyte effector functions are implicated in NCGN. Most of this information is derived from in vitro experiments and the in vivo role needs to be established. For example, active neutrophil serine proteases (NSPs) may modulate the inflammatory response by processing cytokines, growth factors, surface receptors, signaling molecules, and extracellular matrix proteins. It is also conceivable that active NSPs participate in IL-1β generation. PR3, neutrophil elastase (NE) and cathepsin G (CG) are the most abundant NSPs residing in neutrophil granules and monocyte lysosomes. NSP activation requires proteolytic processing by the lysosomal cysteine protease dipeptidyl peptidase I (DPPI). Using our experimental model, we showed that DPPI$^{-/-}$ deficient bone marrow (BM) protected from NCGN. Mice reconstituted with NE$^{-/-}$xPR3$^{-/-}$ BM were equally protected against NCGN and this effect was accompanied by a significantly reduced IL-1β generation in DPPI$^{-/-}$ BM-reconstituted animals. Compared to WT monocytes, DPPI$^{-/-}$ monocytes produced less IL-1β in vitro when stimulated with anti-MPO antibodies. The reduction in IL-1β production was NSP-dependent as exogenous addition of PR3 corrected the defect in DPPI-deficient monocytes. Finally, the specific IL-1 receptor antagonist, anakinra, protected animals against anti-MPO Ab-induced NCGN suggesting that IL-1β is a critical inflammatory mediator in this model. We believe that NSPs and IL-1β may provide novel therapeutic targets for ANCA-mediated diseases in humans.

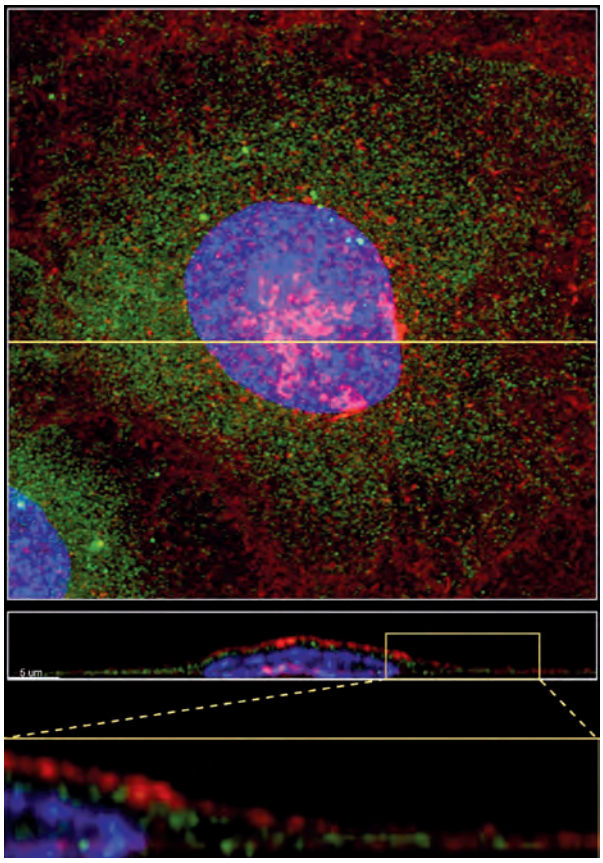
Endothelial cells acquire functional neutrophil proteins

During inflammation activated neutrophils leave the blood stream and migrate towards the inflammatory site. In this process, neutrophils interact with endothelial cells (EC). We speculated that during this process neu-

trophil proteins are transferred to the EC. MPO is one of the most abundant neutrophil proteins. Active MPO has the unique capacity to oxidize chloride ions and utilizes hydrogen peroxide formed during the respiratory burst to generate hypochlorous acid (HOCl), a potent microbicide that contributes to host defense. However, MPO was implicated in the promotion of cardiovascular disease and elevated plasma MPO provides an independent vascular inflammation marker that is linked to increased cardiovascular morbidity and mortality. MPO adversely influences important vascular functions and promotes atherosclerosis. Precisely how the endothelium acquires active MPO from neutrophils was not clear. EC could capture soluble MPO released into the bloodstream by activated neutrophils. We established an additional mechanism where MPO is transferred at intimate neutrophil-EC contacts. This effect was dependent on neutrophil number, exposure time, and incubation temperature. Transfer occurred in several EC types, increased with endotoxin, and was not accompanied by MPO release into the medium. Addition of blocking antibodies against β2 integrins and β2 integrin deficiency abrogated MPO transfer. EC-acquired MPO was enzymatically active, as demonstrated by its ability to oxidize aminophenyl fluorescein in the presence of a hydrogen peroxide source and might therefore participate in vascular damage and dysfunction. We believe that these findings could be therapeutically relevant in atherosclerosis and vasculitis.

Genetic regulation of the PR3 presenting neutrophil surface receptor CD177

PR3 is the major autoantigen in patients with granulomatosis with polyangitis (formerly Wegener's granulomatosis). PR3 is harbored by the entire neutrophil population but is bimodally presented on the neutrophil membrane yielding membrane PR3 $(mPR3)^{low}$ and $(mPR3)^{high}$ subsets. We found in our patient cohort that the larger the $mPR3^{high}$ subset the worse the clinical disease course. From a twin study and a study in HLA matched siblings we learned that the percentage of $mPR3^{high}$ neutrophils is genetically determined with large effects coming from the HLA region. We described neutrophil-specific CD177 previously as a membrane receptor for PR3. CD177 receptor protein and full-length mRNA are expressed by the $CD177^{positive}/mPR3^{high}$ population, but not by the $CD177^{negative}/mPR3^{low}$ neutrophil population. We are now studying the genetic and epigenetic mechanisms that control the restricted CD177 gene expression. We perform DNA and cDNA analysis of the CD177 gene and genome-wide, copy-number variation analysis, haplotype studies in trios, studies on methylation, histone modifications, and non-coding RNAs. We will assess blood- and stem cell-derived human neutrophils.



An endothelial cell has acquired MPO (green) from an adherent neutrophil. The EC nucleus is in blue and the cell membrane in red.

Selected Publications

Jerke, U, Rolle, S, Purfurst, B, Luft, FC, Nauseef, WM, Kettritz, R: b2 integrin-mediated cell-cell contact transfers active myeloperoxidase from neutrophils to endothelial cells. J Biol Chem, 288: 12910-12919, 2013.

Schreiber, A, Pham, CT, Hu, Y, Schneider, W, Luft, FC, Kettritz, R: Neutrophil serine proteases promote IL-1βeta generation and injury in necrotizing crescentic glomerulonephritis. J Am Soc Nephrol. 23: 470-482, 2012.

Jerke, U, Rolle, S, Dittmar, G, Bayat, B, Santoso, S, Sporbert, A, Luft, F, Kettritz, R: Complement Receptor Mac-1 Is an Adaptor for NB1 (CD177)-mediated PR3-ANCA Neutrophil Activation. J Biol Chem, 286: 7070-7081, 2011.

Bontscho, J, Schreiber, A, Manz, RA, Schneider, W, Luft, FC, Kettritz, R: Myeloperoxidase-specific plasma cell depletion by bortezomib protects from anti-neutrophil cytoplasmic autoantibodies-induced glomerulonephritis. J Am Soc Nephrol. 22: 336-348, 2011.

Schreiber, A, Rolle, S, Peripelittchenko, L, Rademann, J, Schneider, W, Luft, FC, Kettritz, R: Phosphoinositol 3-kinase-gamma mediates antineutrophil cytoplasmic autoantibody-induced glomerulonephritis. Kidney Int. 77: 118-128, 2010.

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Photo: David Ausserhofer/MDC

Jeanette Schulz-Menger

Cardiac MRI

The CMR group at the Experimental Clinical Research Center (ECRC) and Clinics for Cardiology and Nephrology of the HELIOS Clinics Berlin Buch has focused research on in vivo assessment of functional and structural myocardial abnormalities related to non-ischemic diseases and ischemic heart disease. Our main hypothesis is, that the in-vivo differentiation of myocardial structural injury is possible in preserved ejection fraction and will help to explain remodeling processes. Furthermore, we understand cardiovascular disease as systemic disorders and aim to understand the mechanism and the interaction of vascular and cardiac disorders. In many projects, we are treading the path from the phantom/model including development of techniques into clinical research. Developing and establishing innovative imaging biomarker will allow for quantification of structural integrity and in-vivo evaluation of prognostic relevant processes.

As Cardiovascular Magnetic Resonance (CMR) has the unique possibility to differentiate myocardial injury, we are focusing on clinical research using a 1.5 clinical MRI-scanner. The group was able to expand the research activities by developing translational research tools applying advanced imaging modalities in close collaboration with the Berlin Ultra-High-Field Facility (B.U.F.F.). As we were a part of the application and establishment process one of the key aspects is cardiovascular research. This could only be established fast, because the head of the Experimental MR-Group (Prof Niendorf) has a strong interest and experience in CMR-research.

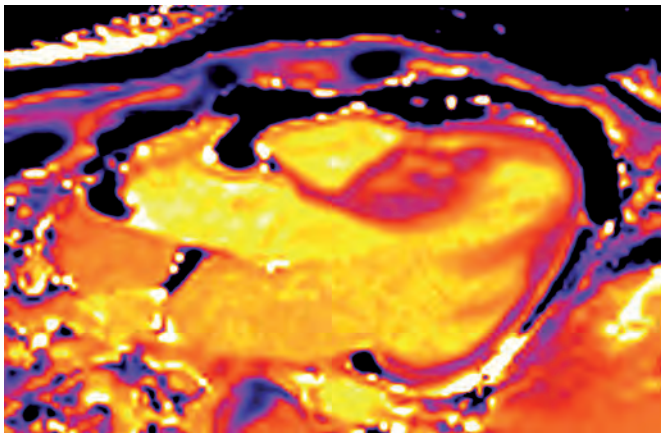
During the last two years we were installing a post-processing lab (CMR reading and development) giving us the opportunity to speed up processes. In 2011 we founded an academic outpatient department of cardiology focused on myocardial disease at the ECRC and established a research database dedicated on patient oriented research.

The group has several outreach activities. Jeanette Schulz-Menger is one of the Principal Investigators of the Charité within the German Center of Cardiovascular Research and is co-heading the Focus Group for Cardiovascular Imaging of the German National Cohort. Furthermore, she was elected as member of the Executive Committee of the Society for Cardiovascular Magnetic Resonance. Florian von Knobelsdorff and she were members of the writing group in societal position statements.

Myocardial tissue injury and integrity

The assessment of myocardial tissue changes during follow-up is an ongoing challenging task in cardiovascular research and clinical cardiology and has been our main focus since years. The in vivo differentiation of myocardi-

Figure 1. *Quantitative assessment of focal fibrosis in hypertrophic cardiomyopathy.*



al structure, cell integrity and identification of (patho-)physiological mechanism is the driving force of our research. CMR has the capability to differentiate between the various forms of myocardial injuries (e.g. edema, hyperemia and fibrosis) already in preserved ejection fraction. During the last years we have prepared the ground to the next step towards quantification of structural changes including assessment of extracellular volume. The underlying approach is parametric mapping. As a first step we published recently (2014) normal values at different field strengths and are currently working on the assessment of non-ischemic heart diseases. We expect, that cell-integrity can be quantified using the wash-out kinetics of contrast-media, but this can be influenced by their specific characteristics. We have faced its potential influence as published in 2013.

Since 2010 (first publication) we have been engaged in myocardial steatosis measured by myocardial triglycerides (MTG) as an influencing factor on myocardial remodeling. We investigated obese otherwise healthy female with preserved ejection fraction. Already in 2011 we could publish an inverse relation between overweight and fitness, followed by 2012 publication on the influence of diet on MTG and the relation between LVH and MTG.

Vascular MR

As mentioned, we are understanding cardiovascular diseases as systemic disorders and so far we have also started work an vascular changes of the great vessels including quantification of wall alteration. Aortic dilatation is a frequent finding in different diseases like arterial hypertension, aortic valve diseases and following aortic valve replacement. Apart from diseases of the aortic wall itself, hemodynamic factors are supposed to promote aortic remodeling. We could establish contrast-media free techniques and applying 4D flow imaging interaction of hemodynamics and remodeling in different aortic valve diseases could be published in 2013. Currently we are working on the identification of prognostic factors for further remodeling.

Cardiac MR at Ultra Highfield

The close collaboration with B.U.F.F. at MDC allowed to publish several joint paper including the worldwide first paper assessing cardiac function of the right ventricle in volunteers at 7.0 Tesla in 2013. This challenging research is ongoing and we expect further results soon.

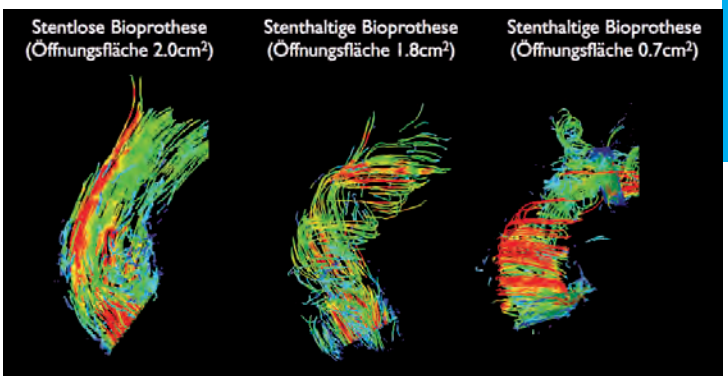


Figure 2. In-vitro *4D flow imaging of heart valve prostheses*

Selected Publications

von Knobelsdorff-Brenkenhoff F, Tkachenko V, Winter L, Rieger J, Thalhammer C, Hezel F, Graessl A, Dieringer MA, Niendorf T, Schulz-Menger J.Assessment of the right ventricle with cardiovascular magnetic resonance at 7 Tesla.J Cardiovasc Magn Reson. 2013 Mar 14;15:23.

Utz W, Engeli S, Haufe S, Kast P, Böhnke J, Haas V, Hermsdorf M, Wiesner S, Pofahl M, Traber J, Luft FC, Boschmann M, Jordan J, Schulz-Menger J.Int J Cardiol. 2013 Aug 10;167(3):905-9. Epub 2012 Apr 10.

Dieringer MA, Hentschel J, de Quadros T, von Knobelsdorff-Brenkenhoff F, Hoffmann W, Niendorf T, Schulz-Menger J. Design, construction, and evaluation of a dynamic MR compatible cardiac left ventricle model. Med Phys. 2012 Aug;39(8):4800-6.

Wassmuth R, Prothmann M, Utz W, Dieringer M, von Knobelsdorff-Brenkenhoff F, Greiser A, Schulz-Menger J.Variability and homogeneity of cardiovascular magnetic resonance myocardial T2-mapping in volunteers compared to patients with edema.J Cardiovasc Magn Reson. 2013 Mar 27;15:27.

Rudolph A, von Knobelsdorff-Brenkenhoff F, Wassmuth R, Prothmann M, Utz W, Schulz-Menger J.J Magn Reson Imaging. 2013 Oct 22. doi: 10.1002/jmri.24264. [Epub ahead of print]

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Photo: David Ausserhofer/MDC

Maik Gollasch

Registry Diabetic Nephropathy / Blood Vessel Function and Target-Organ Damage

Current projects

Our group focuses on ion channels, primarily in vascular smooth muscle cells (VSMC), to clarify mechanisms contributing to hypertension and cardiovascular disease. Potassium channels, chloride channels and transient receptor potential (TRP) channels have received special attention. In collaboration with Björn Schroeder, we investigated the role of smooth muscle TMEM16a chloride channels. We showed that VSMC TMEM16 channels function as regulator of agonist-dependent arterial constriction and systemic blood pressure. We are currently studying angiotensin (Ang) II type 1a receptors (AT1aR) and ryanodine receptor isoforms (Figure 1) in VSMC potassium channel gating and myogenic tone. Michael Bader and Thomas Jentsch are helping us here. We also collaborate with Huang Yu, Hong Kong, China. We found that protein kinase Cdelta plays a pivotal role in angiotensin II-induced endothelial cyclooxygenase-2 expression, which represents a novel pathway to limit vascular inflammation. We also have a project focusing on the perivascular adipose tissue as a source for relaxing factors. Here, we collaborate with Wolf-Hagen Schunck, who has peaked our interests in eicosanoids. Finally, in collaboration with the German Institute of Human Nutrition (DifE), we are studying human diabetic nephropathy with a focus on genetics.

TMEM16a and TRP channels

TMEM16a, TRPC6, and TRPV1 channels are expressed in the vasculature. We used smooth muscle specific TMEM16a deficient mice and found that TMEM16a downregulates agonist-induced vasoconstrictions and thereby contributes to blood pressure regulation. Our current research in this area is directed towards identifying a role of TMEM16a in collateral arterial networks. In collaboration with Ferdinand Le Noble, we have examined the role of the Notch ligand Delta-like 4 (Dll4) in formation of collateral arterial networks and in peripheral ischemia. We found that loss of Dll4 in mice stimulates collateral formation and angiogenesis, but in ischemia such beneficial effects are overruled by adverse vessel functionality. We have also examined TRPV1 and TRPV4 channels in regulating renal blood flow. TRPV1 channels and their natural activator 20-HETE may contribute to ischemia/reperfusion (I/R) -induced kidney injury.

Eicosanoids and inflammation

EETs serve as endothelial-derived hyperpolarizing factors (EDHF), but may also affect cardiovascular function by anti-inflammatory mechanisms. Our current research in this area is directed towards identifying red blood cells as source of vasodilatory EETs and metabolism of eicosanoids by soluble epoxide hydrolase (sEH). We found that variations in the human sEH gene are associated with recurrence of atrial fibrillation after catheter ablation. Hypertension and vascular dysfunction result in the increased expression of COX-2. We identified a novel role of PKCδ in angiotensin II-induced endothelial COX-2 upregulation and release of proatherosclerotic cytokine monocyte chemoattractant protein-1. The findings raise the possibility of curtailing endothelial COX-2 expression as a mean of limiting vascular inflammation. We also found that CLDN3 plays an important role in the passage of leukocytes across the brain vasculature. These findings may lead to new therapies for multiple sclerosis and Alzheimer's disease, in which neuro-inflammation is common.

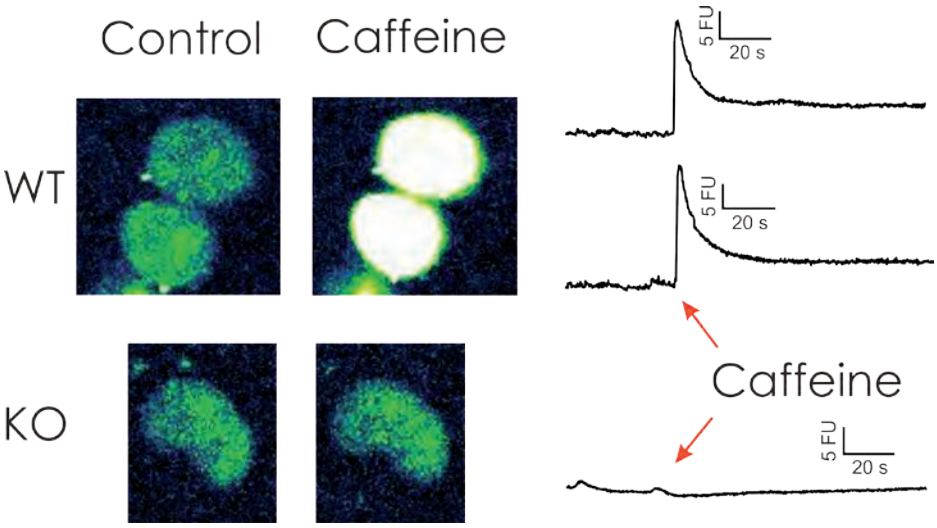


Figure 1. Intracellular release of calcium ions by ryanodine type II receptor calcium channels in mesenteric artery smooth muscle cells. *Intracellular calcium release is visualized by calcium sensitive dyes and Nipkow spinning disc microscopy. Three freshly-isolated cells are shown in this figure: The upper two cells are isolated from wild-type (WT) mice; the lower cell is isolated from a ryanodine receptor type 2 deficient (KO) mouse. The stimulator of intracellular ryanodine receptors caffeine (10 mM) induced a strong calcium release signal in WT, but not in KO cells.*

Vasodilator signals from perivascular adipose tissue

We have identified a vasorelaxing factor produced in the perivascular adipose tissue (ADRF). Our recent work showed that KCNQ channels could represent the subtype of K_v channels involved. The "third gas", namely H_2S, could represent ADRF. However, other adipokines may also play a role. We identified alterations in the paracrine control of arterial tone by periadventitial adipose tissue in animal models of hypertension and metabolic disease. KCNQ and cystathionine gamma-lyase deficient mice are available to us to clarify the role of K_v channels and H_2S. ADRF and its putative targets (KCNQ channels) might represent exciting new targets for the development of drugs for treatment of cardiovascular and metabolic disorders.

Genetic renal diseases

An outgrowth of Maik Gollasch's clinical responsibilities has been a focus on clinical genetics related to renal diseases. We have performed functional analyses of mutations causing familiar kidney diseases with specific emphasis on TRPC6 channels in focal and segmental glomerulosclerosis (FSGS). We found that the proteoglycan syndecan 4 regulates TRPC6 channels in podocytes via RhoA/Rho-associated protein kinase signaling. We are also studying human diabetic nephropathy with a focus on genetics. For these purposes, we recently established an Outpatient Kidney Clinic at Charité Campus Buch and the Registry of Diabetic Nephropathy (http://www.charite-buch.de/rdn/). We are continuously recruiting patients for our Registry and renal/vascular disease-specific family studies. Through these studies, we hope to identify novel mechanisms leading to increased cardiovascular risk and target-organ damage and novel treatment targets.

Primary funding

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Selected Publications

Heinze C, Seniuk A, Sokolov MV, Huebner AK, Klementowicz AE, Szijártó IA, Schleifenbaum J, Vitzthum H, Gollasch M, Ehmke H, Schroeder BC, Hübner CA. Disruption of vascular Ca2+-activated chloride currents lowers blood pressure. J Clin Invest. 2014 Feb 3;124(2):675-86.

Kooij G, Kopplin K, Blasig R, Stuiver M, Koning N, Goverse G, van der Pol SM, van Het Hof B, Gollasch M, Drexhage JA, Reijerkerk A, Meij IC, Mebius R, Willnow TE, Müller D, Blasig IE, de Vries HE. Disturbed function of the blood-cerebrospinal fluid barrier aggravates neuro-inflammation. Acta Neuropathol. 2013 Dec 20. [Epub ahead of print] PubMed PMID: 24356983.

Cristofaro B, Shi Y, Faria M, Suchting S, Leroyer AS, Trindade A, Duarte A, Zovein AC, Iruela-Arispe ML, Nih LR, Kubis N, Henrion D, Loufrani L, Todiras M, Schleifenbaum J, Gollasch M, Zhuang ZW, Simons M, Eichmann A, le Noble F. Dll4-Notch signaling determines the formation of native arterial collateral networks and arterial function in mouse ischemia models. Development. 2013 Apr;140(8):1720-9.

Zavaritskaya O, Zhuravleva N, Schleifenbaum J, Gloe T, Devermann L, Kluge R, Mladenov M, Frey M, Gagov H, Fésüs G, Gollasch M, Schubert R. Role of KCNQ channels in skeletal muscle arteries and periadventitial vascular dysfunction. Hypertension. 2013 Jan;61(1):151-9.

Köhn C, Schleifenbaum J, Szijártó IA, Markó L, Dubrovska G, Huang Y, Gollasch M. Differential effects of cystathionine-γ-lyase-dependent vasodilatory H2S in periadventitial vasoregulation of rat and mouse aortas. PLoS One. 2012;7(8):e41951

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Photo: David Ausserhofer/MDC

Silke Rickert-Sperling

Cardiovascular Genetics

The Sperling Lab focuses on understanding the molecular basis of cardiac dysfunction and cardiovascular disorders, in particular congenital heart malformations. Our team combines molecular biology, bioinformatics and clinical knowledge in systems biology approaches to study cardiac development and muscle maturation in human and mice. We investigate disease-associated genes and epigenetic modulators in the genomic, transcriptomic and proteomic context. We have identified the transcription factor CITED2 as a human disease gene and characterized the chromatin factor DPF3, the first protein shown to bind histone acetylation and methylation marks via its PHD domain. Moreover, we study transcription networks underlying heart development and showed the interplay between multiple cardiac transcription factors and histone modifications. Finally, we demonstrated the polygenic origin of Tetralogy of Fallot by introducing a novel computational approach to identify disease-related genes. Our long-term goal is to combine knowledge of molecular etiologies and mechanisms to improve preventive and therapeutic opportunities for patients with congenital heart diseases.

Cardiac transcription networks

Huanhuan Cui, Vikas Bansal, Katherina Bellmann, Marcel Grunert, Kerstin Schulz

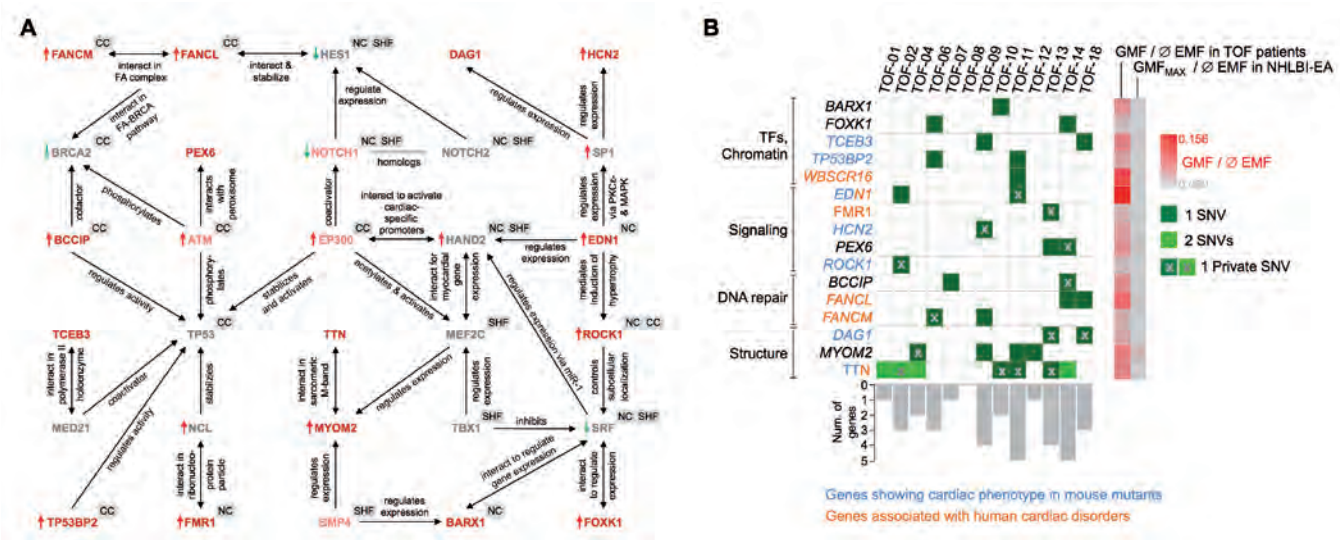
Cardiac and skeletal muscle development is a finely tuned process that requires a precise control of gene expression, which is regulated by the interplay of transcription factors, histone modifications and chromatin remodeling complexes. Studying murine cardiomyocytes, we could show the interdependency of key cardiac transcription factors (Gata4, Mef2a, Nkx2.5, and Srf), activating histone modifications (H3ac, H4ac, H3K4me2, and H3K4me3) and microRNA expression. However, it is widely unknown which impact the exact position of histone marks (relative to the transcription start site) has on cardiac and skeletal muscle gene expression. To elucidate this question, we determined the genomic binding profiles of three histone marks as well as the RNA expression in murine myoblasts and differentiated myotubes. Computational analysis and integration of the data showed that a position-dependent profile of histone modifications marks muscle tissue specific genes, which will hopefully enhance our understanding of muscle maturation.

Chromatin remodeling is essential for regulating the access of the transcription machinery to the DNA. We characterized the epigenetic transcription factor DPF3 (BAF45c), which recruits the BAF chromatin remodeling complex to acetylated as well as methylated histones. Thus, it connects distinct regulatory signals of the histone code and enables a tissues-specific read-out based on its neural, cardiac and muscle specific expression. To further analyze the protein's function, we generated Dpf3 knockout mice. The animals are viable and fertile; they now await in-depth characterization by long-term monitoring, cardiac imaging and expression analysis of Dpf3 downstream targets by RNA sequencing. This will complement our knowledge already gained in zebrafish, where dpf3 is essential for cardiac function and somite structuring, and give novel insights into the transcription networks underlying heart and muscle development.

Congenital Heart Disease

Marcel Grunert, Cornelia Dorn, Vikas Bansal, Andreas Perrot, Sophia Schönhals, Sandra Schmitz, Andrea Behm

Congenital heart diseases (CHD) are the most common birth defect in human and affect nearly 1% of all newborns. They are a het-



Interaction and functions of significantly over-mutated TOF genes identified from targeted re-sequencing. **(A)** *TOF genes (dark red) interact in a molecular network with other genes of potential interest (light red and gray). Several genes show individual expression disturbances (red and green arrows).* **(B)** *Multiple TOF genes are affected by rare and/or private variations in TOF patients. The GMF is indicated in comparison to control individuals (European Americans from the Exome Sequencing project of the NHLBI).*

erogeneous group of disorders ranging from minor alterations to complex malformations requiring multiple surgeries. The majority of CHDs are probably caused by a combination of multiple genetic, epigenetic and environmental factors. The identification of disease-related genes has been a great challenge and is complicated by the fact that every healthy human carries hundreds of probably damaging genomic variations that seem to be tolerated in the individual context.

We aimed to unravel the complex genetics of Tetralogy of Fallot (TOF), the most common cyanotic form of CHD, and to develop novel methods for the identification of disease-related genes. Focusing on single nucleotide variations, we developed the novel concept of the gene mutation frequency (GMF), which considers all deleterious variations in a gene and can determine over-mutated genes in a patient cohort in comparison to control individuals. This provided strong evidence for the polygenic origin of TOF and identified 16 significantly over-mutated genes affected by combinations of deleterious private and rare mutations. The genes interact in a molecular network and show sustained expression in the adult heart, which might help to understand differences in long-term clinical outcomes of TOF patients. Moreover, we developed a novel method to identify copy number variations (CNVs) that can be applied to small cohorts and could identify CNVs affecting important cardiac regulators in TOF patients.

To further study the underlying etiology of TOF, the establishment of patient-specific induced pluripotent stem cells (iPSCs) and their differentiation into cardiomyocytes will be a valuable model. Moreover, we aim to integrate additional genetic and epigenetic data, which will hopefully enable a more comprehensive understanding of essential disease mechanisms.

Selected Publications

Grunert M*, Dorn C*, Schueler M, Dunkel I, Schlesinger J, Mebus S, Alexi-Meskishvili V, Perrot A, Wassilew K, Timmermann B, Hetzer R, Berger F, Sperling SR (* contributed equally). Rare and Private Variations in Neural Crest, Apoptosis and Sarcomere Genes Define the Polygenic Background of Isolated Tetralogy of Fallot. Hum Mol Genet. 2014 Jan 23. [Epub ahead of print]

Bansal V*, Dorn C*, Grunert M, Klaassen S, Hetzer R, Berger F, Sperling SR (* contributed equally). Outlier-Based Identification of Copy Number Variations Using Targeted Resequencing in a Small Cohort of Patients with Tetralogy of Fallot. PLoS One. 2014 Jan 6;9(1):e85375.

Dorn C*, Grunert M*, Sperling SR (* contributed equally). Application of high-throughput sequencing for studying genomic variations in congenital heart disease. Brief Funct Genomics. 2014 Jan;13(1):51-65.

Schlesinger J*, Schueler M*, Grunert M*, Fischer JJ*, Zhang Q, Krueger T, Lange M, Tönjes M, Dunkel I, Sperling SR (* contributed equally). The cardiac transcription network modulated by Gata4, Mef2a, Nkx2.5, Srf, histone modifications, and microRNAs. PLoS Genet. 2011 Feb;7(2):e1001313.

Lange M, Kaynak B, Forster UB, Tönjes M, Fischer JJ, Grimm C, Schlesinger J, Just S, Dunkel I, Krueger T, Mebus S, Lehrach H, Lurz R, Gobom J, Rottbauer W, Abdelilah-Seyfried S, Sperling S. Regulation of muscle development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex. Genes Dev. 2008 Sep 1;22(17):2370-84.

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Endocrinology, Diabetes and Nutritional Medicine

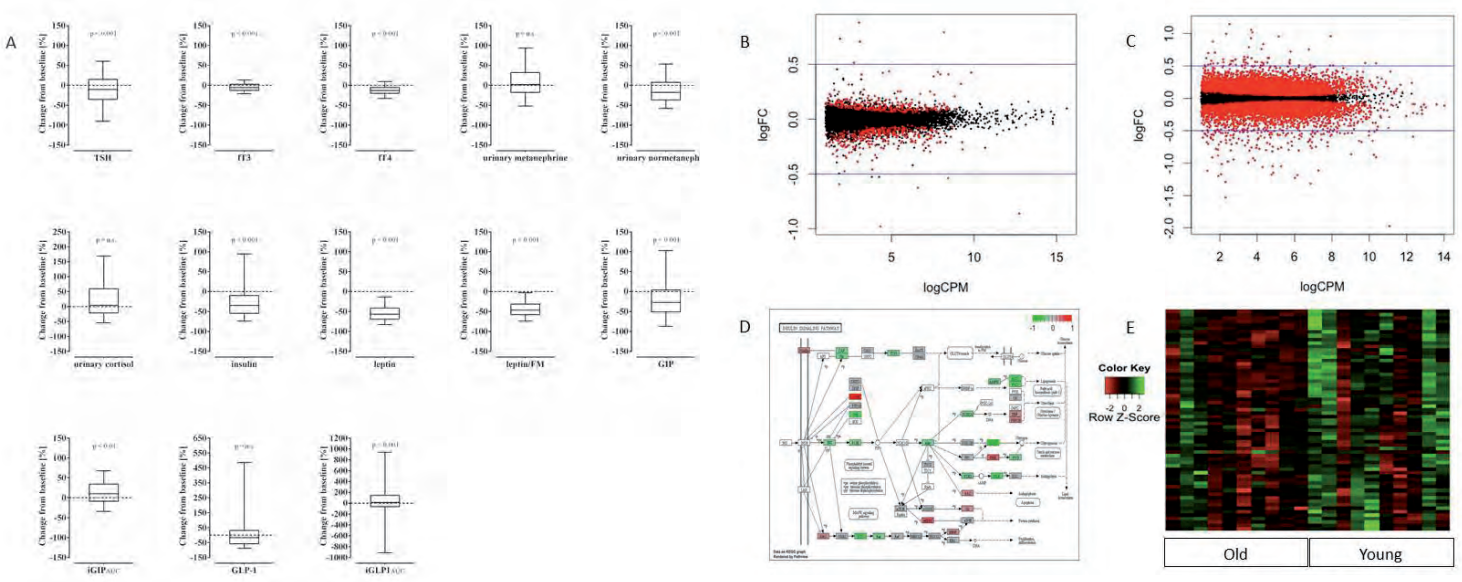
Physical inactivity contribute to the projection that cardiovascular disease but also cancer will remain among the major causes of death in Germany. Against the background of an individual genetic make-up, environmental risk factors interact with metabolic/endocrine pathways to finally modify disease risk. Apparently, the endogenous reaction to environmental stimuli depends on numerous non-modifiable factors such as age, gender, but also stage of disease and existing co-morbidities. Thus, an age-related metabolic inflexibility to exogenous stimuli is likely to contribute to metabolic and cardiovascular ageing. We therefore aim to identify novel and further elucidate known endogenous metabolic modifiers of cardio-metabolic health. We analyze the interrelation between environment, specific molecular targets and cardiometabolic risk in relation to age and comorbidities to finally translate those findings into preventive practice. The group interacts with several other research groups in Berlin and outside and is partner of several joint research projects from the DFG, BMBF and EU.

Hormonal regulation of energy expenditure and metabolism

Weight loss substantially improves several cardiovascular risk factors and the benefit appears to persist as long as weight reduction is maintained. However, mostly weight regain is observed during follow-up. This seems to be mediated by a coordinated endocrine response, including several adipokines and myokines like leptin and myostatin, and numerous other hormones like insulin, thyroxin and inkretines like GLP-1 - factors crucially involved in the regulation of food intake, muscle mass and metabolism, energy expenditure, and physical activity. To understand the role of this complex network is a major research issue of the group. We aim to elucidate the relevance of those neuroendocrine circuits by delineating the effects on metabolism and cardiovascular risk. J Spranger is speaker of a DFG-funded clinical research group (KFO218/2) being devoted to investigate the endocrine regulation of body weight maintenance. This research group was excellently evaluated and is currently in its second funding period. Among others we currently perform a large human intervention trial investigating the relation of lifestyle to body weight maintenance. In collaboration with other partners from the MDC and Charité, we study hormonal regulators of energy metabolism by analyzing the relevance of novel hormones such as thyronamines, but also the impact of estrogen receptor signalling and the role of miRNAs. Together with partners from the Charité Center for Advance Neuroimaging and the Bernstein Institute of Computational Neuroscience, we study behavioural measures associated to central-nervous reward systems using fMRI technology.

Metabolism and peripheral tissues

We are particularly interested in tissue specific processes. Even if the role of adipose and muscle tissue is well established regarding selected cytokines, the complex regulation and interaction in adipose and muscle tissue is still not fully understood. Using in vivo and ex vivo approaches we analyse tissue specific hormonal and metabolic pro-



The group investigates weight loss induced changes of hormonal circuits and gene expression in different tissues and analyses the impact on long term body weight and health. Perturbation of neuroendocrine axes during weight loss **(A)**. *Differentially expressed genes in skeletal muscle* **(B)** *and adipose tissue* **(C)** *during a 12-weeks weight loss. logFC indicates log fold change of differentially expressed genes (red points), logCPM indicates log of counts per million, black points indicate not differentially expressed genes.* **(D)** *Pathway analyses: weight loss induced changes of insulin signaling pathway in adipose tissue of obese subjects after weight loss (n=87). Green color reflects downregulation of gene transcription and red color reflects upregulation of gene transcription during weight loss. Age dependend regulation of gene expression and the impact on metabolic and cardiovascular health is adressed by the group. Exemplarily weight loss induced changes of transcription of gene involved in lipid metabolism is different in old and young individuals in adipose tissue* **(E)**.

cesses in human adipose and muscle tissue and their role in body weight regulation and energy metabolism. Exemplarily, we performed a RNASeq analyses of fat and muscle biopsies of about 80 individuals before and after dietary weight loss. These individuals were observed for another two years to evaluate the predictors of body weight maintenance. We found a weight loss induced regulation of numerous signalling pathways in fat but also muscle. Whether those weight loss mediated changes are essential for long term outcome and cardiovascular health is addressed in our current studies. The impact of weight loss on skeletal muscle is addressed by the group, as decrease of muscle mass is regularly observed during weight reduction and might be important for long term failure of most lifestyle interventions. Within this project we also aim to separate effects of modified body composition and of acute negative energy balance on local and systemic metabolism. This project is also investigated within a collaborative research effort of the ECRC on skeletal muscle. We are especially interested in lifestyle induced changes and the impact on metabolism and long term cardiovascular health. We currently investigate the role of salt (sodium) on metabolism together with a number of ECRC researchers. Those findings may finally help to better understand which environmental parameters affect cardio-metabolic risk.

Selected Publications

Dimas AS, Lagou V, Barker A, Knowles JW, Mägi R, Hivert MF, Benazzo A, Rybin D, Jackson AU, Stringham HM, Song C, Fischer-Rosinsky A, Boesgaard TW, Grarup N, Abbasi FA, Assimes TL, Hao K, Yang X, Lecoeur C, Barroso I, Bonnycastle LL, Böttcher Y, Bumpstead S, Chines PS, Erdos MR, Graessler J, Kovacs P, Morken MA, Narisu N, Payne F, Stancakova A, Swift AJ, Tönjes A, Bornstein SR, Cauchi S, Froguel P, Meyre D, Schwarz PE, Häring HU, Smith U, Boehnke M, Bergman RN, Collins FS, Mohlke KL, Tuomilehto J, Quertemous T, Lind L, Hansen T, Pedersen O, Walker M, Pfeiffer AF, Spranger J, Stumvoll M, Meigs JB, Wareham NJ, Kuusisto J, Laakso M, Langenberg C, Dupuis J, Watanabe RM, Florez JC, Ingelsson E, McCarthy MI, Prokopenko I; on behalf of the MAGIC investigators. Impact of type 2 diabetes susceptibility variants on quantitative glycemic traits reveals mechanistic heterogeneity. Diabetes. 2013 Dec 2.

Weygandt M, Mai K, Dommes E, Leupelt V, Hackmack K, Kahnt T, Rothemund Y, Spranger J, Haynes JD. The role of neural impulse control mechanisms for dietary success in obesity. Neuroimage. 2013 Dec;83:669-78.

Habegger KM, Stemmer K, Cheng C, Müller TD, Heppner KM, Ottaway N, Holland J, Hembree JL, Smiley D, Gelfanov V, Krishna R, Arafat AM, Konkar A, Belli S, Kapps M, Woods SC, Hofmann SM, D'Alessio D, Pfluger PT, Perez-Tilve D, Seeley RJ, Konishi M, Itoh N, Kharitonenkov A, Spranger J, DiMarchi RD, Tschöp MH. Fibroblast growth factor 21 mediates specific glucagon actions. Diabetes. 2013 May;62(5):1453-63.

Weber-Carstens S*, Schneider J*, Wollersheim T, Assmann A, Bierbrauer J, Marg A, Al Hasani H, Chadt A, Wenzel K, Koch S, Fielitz J, Kleber C, Faust K, Mai K, Spies CD, Luft FC, Boschmann M, Spranger J*, Spuler S* (*shared authorship). Critical illness myopathy and GLUT4: significance of insulin and muscle contraction. Am J Respir Crit Care Med. 2013 Feb 15;187(4):387-96.

Bobbert T, Schwarz F, Fischer-Rosinsky A, Pfeiffer AF, Möhlig M, Mai K, Spranger J. Fibroblast growth factor 21 predicts the metabolic syndrome and type 2 diabetes in Caucasians. Diabetes Care. 2013 Jan;36(1):145-9.

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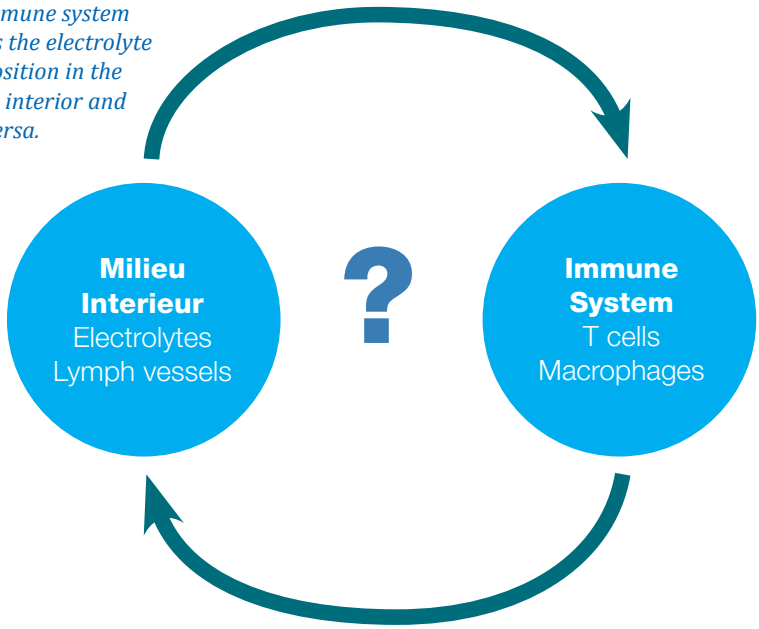


Ralf Dechend

Hypertension-induced Target Organ Damage

The group's major research interests are the renin-angiotensin system, the immune system, and how both systems cause hypertension-induced and Ang II-related target-organ damage. Recent work has extended the concept analyzing how epigenetic factors such as a high-salt microenvironment influences immune cells, hypertension-induced target organ damage, and autoimmunity. In a translational approach, our group focuses primarily on the placenta, heart and kidneys. The group has also been a resource for young clinicians and doctoral students beginning their careers in experimental cardiovascular research

We hypothesise that the immune system affects the electrolyte composition in the milieu interior and vice versa.



The immune system, salt and hypertension-induced target organ damage

Hypertension induces target-organ damage; however, the mechanisms are unclear. We hypothesize that immunological processes are important. In general, the immune system is traditionally considered to be a complex biological system that wards off disease, typically by fighting the invasion of foreign microorganisms. Angiotensin (Ang) II induces inflammation, fibrosis, and growth remodeling via the AT1 receptor. Innate immunity is pivotal in Ang II-related target-organ damage. T cells, macrophages, and dendritic cells all harbor the AT1 receptor. Ang II stimulates T cell proliferation and dendritic cell migration. We found that regulatory T cells modulate Ang II-induced target-organ damage to a point of therapeutic utility. We also have shown that IFN-g signaling plays a pivotal role in cardiac and renal damage.[1]

Together with Jens Titze and his laboratory (Erlangen/Vanderbilt), we provided evidence that sodium, a known risk factor for cardiovascular disease, interacts with the immune system. Recent evidence indicates that cells of the immune system may also have an important function in regulating the electrolyte content of the interstitium, or "milieu intérieur". Current teaching suggests that electrolyte balance in the microenvironment bathing all cells is controlled by isosmotic passive equilibration with plasma. However, recent evidence suggests that this assumption should be revised. Using dietary salt (NaCl) loading studies, it has been shown that water free Na^+ was stored in the skin, resulting in a hypertonic microenvironment in the skin despite stable isotonic conditions in the circulation.[2] Mononuclear phagocytic system (MPS) cells play a pivotal role in regulating the homeostatic processes necessary to allow the body to store Na^+ in the skin.[2] Upon excess

dietary sodium, Na^+ accumulates in the skin and activate the osmotic stress transcription factor, TonEBP (also known as NFAT5) in MPS cells.[2] This activation subsequently results in vascular endothelial growth factor-C (VEGF-C) mediated lymphatic vessel hyperplasia, promoting clearing of hypertonic fluid from the interstitium, and thus, a return to normal levels of interstitial salt. Disturbance in this macrophage-driven regulatory axis therefore results in the accumulation of Na^+ bound in part to glycosaminoglycans within the microenvironment.[2] Subsequently, the consequence of this increased concentration of interstitial Na^+ may be a disturbance in the function of cells residing within this compartment, including infiltrating immune cells. Increased NaCl concentrations induced a 10-fold increase in the cytokine-driven TH17 activation in human and murine naïve T cells.[3] High salt activated the p38/MAPK pathway during cytokine-induced TH17 polarization in these cells, involving TonEBP and the serum- and glucocorticoid-inducible kinase 1 (SGK1). The outcome of this increased TH17 activation resulted in an accelerated and more severe autoimmune disease (multiple sclerosis) in mice on a high-salt diet.[3] The unique global research hypothesis of our laboratory is that: (i) immune cells are essential for the normal regulation of electrolyte concentrations within the microenvironments of the body and that (ii) disturbance of this axis results in an accumulation of interstitial sodium, subsequently affecting the function of these same immune cells (Figure).

Pathogenesis of preeclampsia

Preeclampsia (PE) is a leading cause of maternal death, and perinatal morbidity and mortality resulting from premature delivery and intrauterine growth restriction (IUGR). New hypertension and proteinuria arises secondary to diffuse maternal endothelial and inflammatory dysfunction. We have studied the role of immunological dysregulation in the cause of preeclampsia.[4] We found that activating autoantibodies against the AT1-receptor are implicated in the pathogenesis of preeclampsia. Recently, we showed that they are involved in dysregulating various aspects of the immune system in preeclampsia. We performed gene arrays from preeclamptic and control placentas and identified the CYP subfamily 2J polypeptide 2 (CYP2J2) as a novel candidate gene.[5] CYP2J2 metabolites, 5,6 epoxyeicosatrienoic acids (5,6-EET), were upregulated before the onset of the disease. Cyp P450 inhibition reduced preeclamptic phenotype in two rat models of preeclampsia. 5,6-EET can be metabolized to a thromboxane analog, making it an interesting candidate for mediating preeclampsia. Together with Zsuzsanna Izsvak we are using the sleeping-beauty transposon technique to overexpress candidate genes, such as CYP2J2 specifically in trophoblasts. We are also currently investigating the macrophage – trophoblast interaction, the role of uterine NK cell depletion and upregulation of regulatory T cells on the preeclamptic phenotype. In collaboration with Michael Bader we investigated an inducible transgenic rat model for diabetes mellitus based on shRNA-mediated gene knockdown of the insulin receptor during pregnancy. Using different crosses we can differentiate between placental and fetal insulin resistance with our without maternal insulin resistance. Various degree of IUGR is present in the fetus. In clinical studies, we began to investigate physiological parameters and novel biomarkers during and after preeclampsia, which help to explain the increased cardiovascular risk of former preeclamptic patients.

Patents / Patent applications

Use of renin inhibitors for the prevention or treatment of diastolic dysfunction or diastolic heart failure. 10008427.6-1216, 2005. Inventors: Feldman DL, Luft FC, Mueller DN, Webb R.

Novel Eicosanoid Derivatives. EP-1874, 2009. Inventors: Schunck WH, Wallukat G, Schmidt C, Fischer R, Muller DN (MDC), Puli N and Falck JR (UT Southwestern, Dallas); WO 2010/081683 A1

Selected Publications

1. Markó L, Kvakan H, Park J-K, Qadri F, Spallek B, Binger KJ,Bowman EP, Kleinewietfeld M, Fokuhl V,Dechend R, Muller DN. Interferon gamma (Ifn-γ) signaling inhibition ameliorates Ang II-induced cardiac damage Hypertension 2012;60(6):1430-6.
2. Wiig H, Schröder A, Neuhofer W, Jantsch J, Kopp C, Karlsen TV, Boschmann M, Goss J, Bry M, Rakova N, Dahlmann A, Brenner S, Tenstad O, Nurmi H, Mervaala E, Wagner H, Beck FX, Müller DN, Kerjaschki D, Luft FC, Harrison DG, Alitalo K, Titze J. Immune cells control skin lymphatic electrolyte homeostasis and blood pressure. J Clin Invest. 2013;123(7):2803-15
3. Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Linker RA, Müller DN*, Hafler DA*. Sodium Chloride Drives Experimental Autoimmune Disease by the Induction of Pathogenic Th17 Cells. Nature 2013; 496(7446):518-22 (* shared authorship)
4. Freitag N, Tirado-Gonzalez I, Barrientos G, Herse F, Thijssen VL, Weedon-Fekjaer SM, Schulz H, Wallukat G, Klapp BF, Nevers T, Sharma S, Staff AC, Dechend R and Blois SM. Interfering with Gal-1-mediated angiogenesis contributes to the pathogenesis of preeclampsia. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:11451-6.
5. Herse F, Lamarca B, Hubel CA, Kaartokallio T, Lokki AI, Ekholm E, Laivuori H, Gauster M, Huppertz B, Sugulle M, Ryan MJ, Novotny S, Brewer J, Park JK, Kacik M, Hoyer J, Verlohren S, Wallukat G, Rothe M, Luft FC, Muller DN, Schunck WH, Staff AC and Dechend R. Cytochrome P450 subfamily 2J polypeptide 2 expression and circulating epoxyeicosatrienoic metabolites in preeclampsia. Circulation. 2012;126:2990-9.

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Video:
Can we predict and prevent metastasis in colon cancer?

Translational Oncology of Solid Tumors

Our translational concepts in cancer research aim at the identification of key molecules in tumor progression and metastasis for improvement of prognosis and therapy of solid tumors. We found novel genes, revealed their potential as biomarkers and gained insights in their function within cancer signaling networks. Our main focus is on the metastasis inducers MACC1 and S100A4 as prognostic and predictive biomarkers for colorectal cancer (CRC) and further solid tumors. Our knowledge on these molecular targets is employed for innovative signaling-based approaches to intervene in metastasis. Preclinically validated therapeutic strategies are currently translated into clinical trials to treat patients with metastatic disease.

MACC1: A driver for tumorigenesis and metastasis and a therapeutic target for metastasis restriction in solid cancers

U. Stein, W. Walther, M. Juneja, C. Lemos, P.M. Schlag; in cooperation with W. Birchmeier, G. Dittmar, I. Fichtner, B. Jerchow, H. Schmidt, (MDC), J. von Kries (FMP), U. Rohr (Hoffmann-La Roche)

We discovered the gene metastasis-associated in colon cancer 1 (MACC1) able to induce proliferation and motility *in vitro* and tumor growth and metastasis in mice. MACC1 controls transcriptionally target genes, e.g. Met, promoting cell motility and metastasis. shRNA acting on MACC1, Met, or further MACC1 targets decreased metastases formation. Transgenic vil-MACC1 and vil-MACC1/APC^{min} mice have been generated; MACC1 knock out models are in progress.

We identified the MACC1 gene promoter, and employed it in high throughput screens for first identification of FDA-approved MACC1 inhibitors. We found MACC1 protein binding partners by mass spectrometry essential for MACC1 function and prognostic for CRC patients. We also identified kinases for MACC1 phosphorylation and used specific kinase inhibitors for restriction of MACC1-induced metastasis in mice. MACC1 is a biomarker for disease prognosis and prediction of therapy response for CRC and is confirmed for a variety of solid cancers, linked to patient survival. A first clinical study for MACC1-based stratification of stage II CRC patients for adjuvant therapy was recently completed successfully (with Hoffmann-La Roche).

S100A4: Inhibition of CRC metastasis by Wnt/ß-catenin-pathway-oriented small molecules targeting the metastasis progressor S100A4

U. Stein, W. Walther, U. Sack, M. Dahlmann, P.M. Schlag; in cooperation with I. Fichtner (MDC), R.H. Shoemaker, P. Pandalai (NCI/NIH, Bethesda)

We identified the metastasis gene S100A4 as transcriptional ß-catenin target. S100A4 strongly increased cell motility in vitro and metastasis in mice. In clinical specimens, we demonstrated the prognostic value of S100A4 in primary CRC for metastasis and patient survival. We targeted S100A4 for metastasis restriction in mice, e.g. by systemic application of shRNA acting on S100A4. We identified small molecule transcriptional inhibitors (e.g. niclosamide, calcimycin) from high-throughput screens of pharmacologically active compounds that inhibit S100A4 by intervening in the Wnt/ß-catenin cascade. We also used compounds known to interfere with this pathway (e.g. sulindac). These S100A4 inhibitors reduced migratory and invasive abilities, and restricted metastasis in mice. Now we are translating our findings on S100A4-specific metastasis inhibition into clinical practice. Our assay for circulating S100A4 transcripts in patient blood is used to monitor treatment success. Currently, FDA-approved inhibitory compounds are being tested in clinical phase II trials for metastasized CRC patients: for sulindac, with the NCI/NIH(NCT01856322); for niclosamide, with the Charité, Berlin.

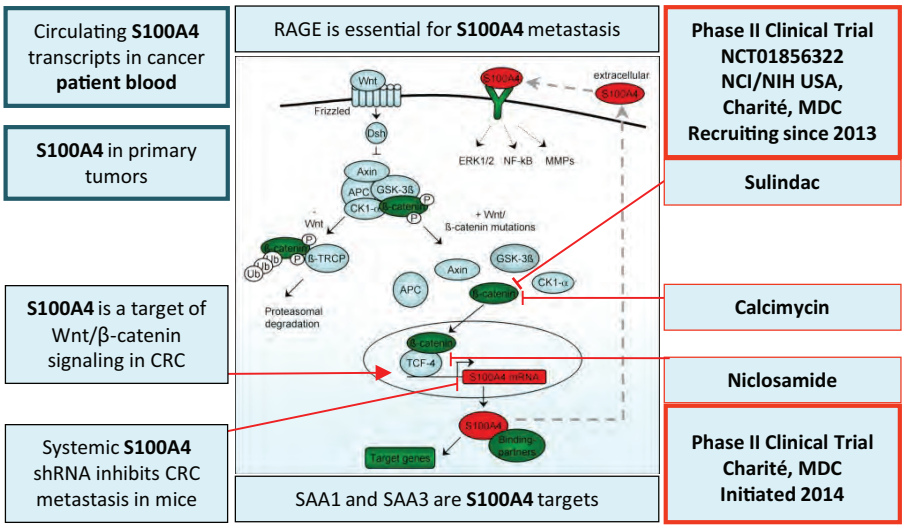


Figure 1. *We demonstrated the prognostic value of the metastasis inducer S100A4 for CRC metastasis linked to patient survival when determined in tumors or blood. We discovered S100A4 as target of the Wnt/ß-catenin pathway, identified the receptor RAGE to be essential for S100A4-induced cell motility, and linked inflammation and metastasis with SAAs as targets of S100A4. Targeting S100A4 with S100A4-shRNA or small molecule inhibitors intervening in Wnt/ß-catenin signaling such as sulindac, calcimycin and niclosamide restricted S100A4-induced metastasis in mice. Clinical phase II trials translating this knowledge for sulindac and niclosamide to treat metastasized CRC patients are currently underway.*

Imaging of carcinomas using a novel RNAi-based detection mechanism

W. Kemmner; in cooperation with B. Ebert (PTB), S. Lin (Guangzhou University, China), R. Zeisig (EPO), R. Haag (Free University Berlin)

In previous studies, we found a significant down-regulation of Ferrochelatase (FECH) expression in various carcinomas leading to the accumulation of the fluorescent metabolite of heme synthesis protoporphyrin IX (PpIX). FECH silencing by folate-PEG cationic lipoplex-encapsulated FECH-siRNA enabled a sensitive in vivo tumor imaging. In the future, FECH-siRNA nanocarriers could be used for sensitive detection of small metastatic tumor clusters. At the same time this technology can be used for photodynamic depletion of tumor cells of primary carcinoma and metastases.

Nonviral gene therapy for solid tumors

W. Walther, D. Kobelt, F. Chiola, P. M. Schlag, U. Stein; in cooperation with F. Kiecker, U. Keilholz, J. Piontek (Charité), I. Fichtner (MDC), B. Wittig (Free University Berlin), M. Schroff, M. Schmidt (MOLOGEN)

A new phase I clinical trial is conducted to evaluate safety and efficiency of nonviral gene therapy using the TNF-α expressing minimalistic MIDGE-vector in patients with in-transit skin metastasis from melanoma (EudraCT: 2007-000423-18). This dose-escalation study will analyze TNF-α expression efficiency after intratumoral jet-injection gene transfer. The safety of the expressed TNF-α and of the vector DNA will be evaluated.

Our efforts also aim at development of novel therapeutic genes. The use of bacterial toxins is an applicable option for treatment of solid tumors. The Clostridium perfringens enterotoxin (CPE) is a pore-forming bacterial toxin, which specifically binds to claudin-3 and -4 and triggers membrane pore formation leading to rapid cell death. We successfully exploit this for selective tumor cell killing by CPE gene therapy of human claudin-3 and -4 overexpressing tumors in mice.

Patents / Patent applications

Stein U, Lederer A, Schlag PM, Herrmann P, Seehofer D. MACC1 as a prognostic biomarker in hepatobiliary tumors Patent application 2013; US 61/829,669

Stein U, Schwabe H, Walther W, Schlag PM. 7a5/Prognostin and use thereof for the diagnostic and therapy of tumors
Patent, granted 2013 EP 1646649B1; 2013 CA 2,526,240; 2011 JP 51-9895; 2011 AU 2004259281; 2010 US 7,851,168 B2

Stein U, Walther W, Sack U, Scudiero D, Schlag PM, Shoemaker RH. Niclosamide for treatment of cancer metastasis
Patent application 2013 PCT/US 14/112,521; 2012 PCT/EP2012/057049; 2011 EP 11162875.6

Walther W, Kobelt D, Schlag PM, Schmidt M. Chemosensitivierung von Melanomen mittels Gentransfer eines TNF-alpha exprimierenden minimalistischen MIDGE-hTNF Vektors.
Patent application 2010 EP 10195929.4

Stein U, Herrmann P, Burock S, Schlag PM. Use of metastasis progressor S100A4 transcripts in body fluids of colorectal and gastric cancer patients.
Patent application 2010 PCT/EP2010/001891; 2009 US 61/261,017

Selected Publications

Stein U, Walther W, Arlt F, Schwabe H, Smith J, Fichtner I, Birchmeier W, Schlag PM. MACC1, a newly identified key regulator of HGF/Met signaling, predicts colon cancer metastasis. Nature Med. 2009, 15:59-67.

Juneja M, Ilm K, Schlag PM, Stein U. Promoter identification and transcriptional regulation of the metastasis gene MACC1 in colorectal cancer. Mol Oncol. 2013, 7:929-43.

Sack U, Walther W, Scudiero D, Selby M, Kobelt D, Lemm M, Fichtner I, Schlag PM, Shoemaker RH, Stein U. Novel effect of anti-helmintic niclosamide on S100A4-induced metastasis in colon cancer. J Natl Cancer Inst. 2011, 103:1018-36.

Dahlmann M, Sack U, Herrmann P, Lemm M, Fichtner I, Schlag PM, Stein U. Systemic shRNA mediated knock down of S100A4 in colorectal cancer xenografted mice reduces metastasis formation. Oncotarget 2012, 3:783-97.

Kobelt D, Aumann J, Schmidt M, Wittig B, Fichtner I, Behrens D, Lemm M, Freundt G, Schlag PM, Walther W. Preclinical study on combined chemo- and nonviral gene therapy for sensitization of melanoma using a human TNF-alpha expressing MIDGE DNA vector. Mol Oncol. 2014 [Epub ahead of print]

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Technology Platforms

Central Technology Platforms

Central Technology Platforms and Core Facilities

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Bettina Purfürst
Electron Microscopy

Gunnar Dittmar
Mass Spectrometry

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Pathophysiology

Hans-Peter Rahn
Preparative Flow Cytometry

Ralf Kühn (from May 2014)
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Norbert Hübner
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Udo Heinemann
Protein Sample Production Facility*

* See group pages N. Hübner (Cardiovascular and Metabolic Disease) and U. Heinemann (Cancer Research)

BIMSB Technology Platforms

Altuna Akalin
Bioinformatics

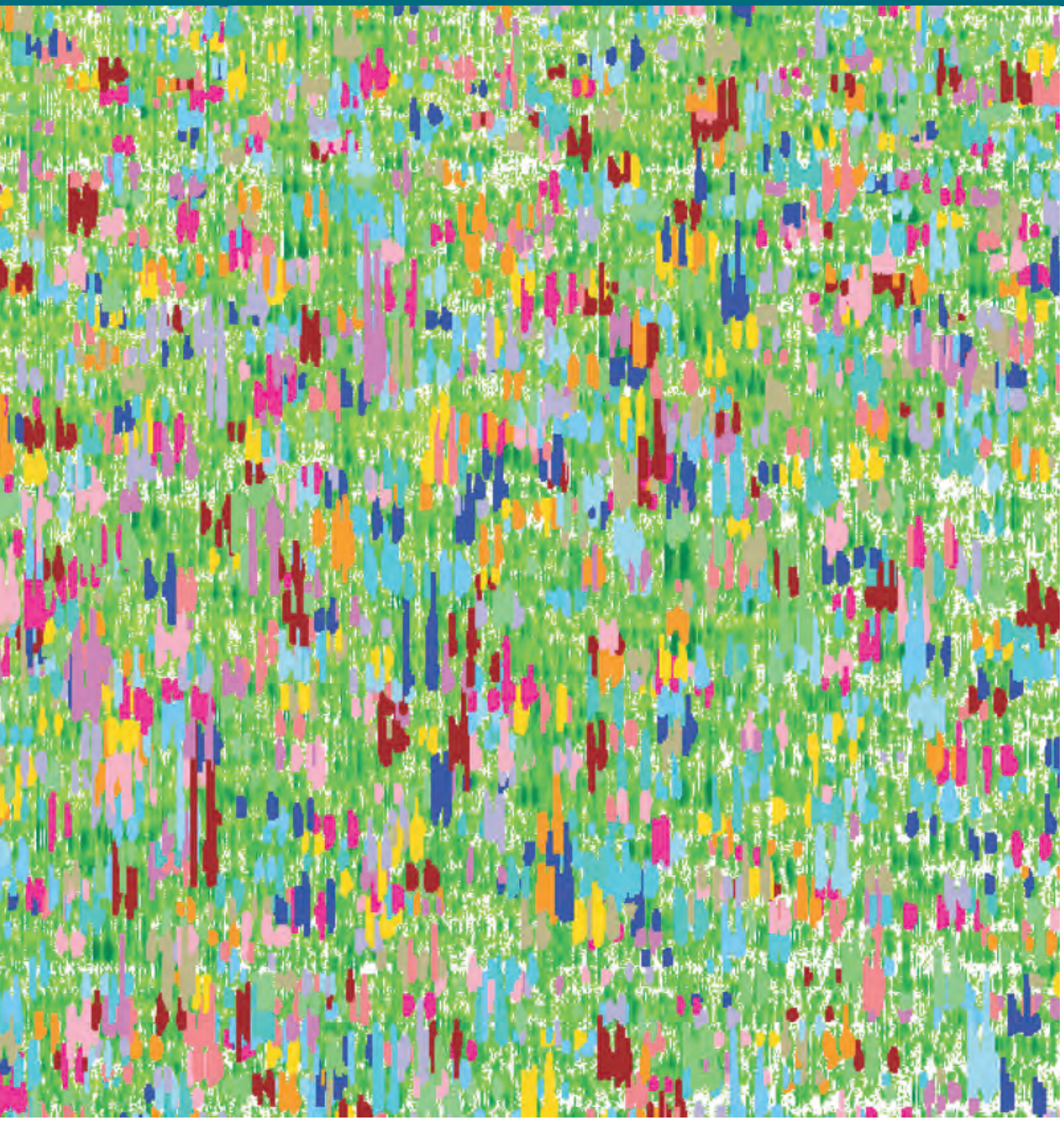
Wei Chen
Genomics

Stefan Kempa
Proteomics / Metabolomics

The Max Delbrück Center has several dedicated Core Facilities and Technology Platforms providing MDC groups (and external users by special request) with facilities and support for a wide range of different techniques. Modern technology and services which would otherwise demand an extremely long and intensive period of training, as well as large investments, are the mainstay of the MDC's central Technology Platforms. These facilities provide all users with the opportunity to acquire both up-to-date service support and the latest scientific know-how.

Additionally, there are technology platforms associated with research groups providing services for Interactomics, magnetic resonance (B.U.F.F.), Microarrays and Protein Sample Production Facility. On the following pages you will find more in depth descriptions of our central Technology Platforms and Core Facilities, the research group associated technology platforms as well as of BIMSB Technology Platforms.

Protein fragments of colon bacteria, analyzed by mass spectrometry.
Proteinfragmente eines Darmbakteriums, analysiert mithilfe eines Massenspektrometers.



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Photo: David Ausserhofer/MDC

Anje Sporbert

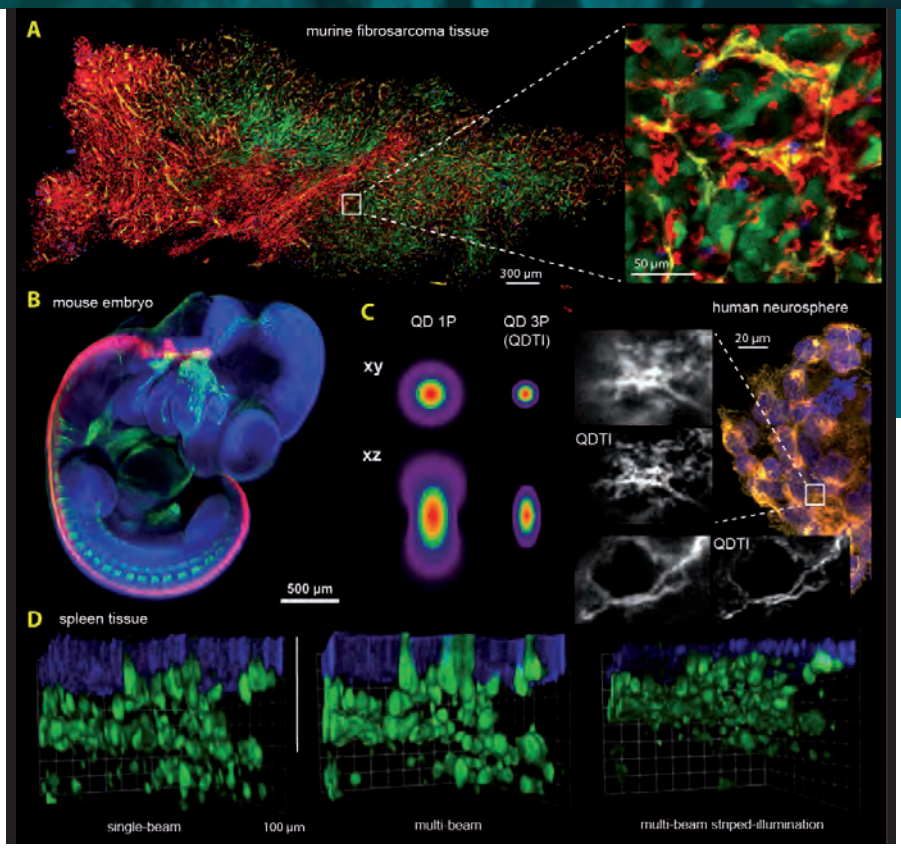
Advanced Light Microscopy

The Advanced Light Microscopy platform offers scientists access to state of the art, high-end imaging techniques and supports the research projects of scientific groups with customised, methodological expertise.
We are currently supporting about 150 researchers from more than 35 groups mainly of the MDC, BIMSB and ECRC.
In addition to teaching scientists in the theoretical aspects of light microscopy, we offer them profound practical training and assure the optimal performance of the provided instrumentation and infrastructure.
In collaborative imaging projects we offer individual user support covering a wide range of expertise, e.g. project planning, optimisation of sample preparation and image acquisition conditions, as well as a customised image analysis.

Available imaging techniques and selected projects

We currently host 10 high-end microscope systems, covering confocal laser scanning, 2-photon, wide-field fluorescence, TIRF and light-sheet based microscopy, as well as laser-assisted micro-dissection. This instrumentation offers advanced imaging techniques for both fixed and live specimen, ranging from cells, tissue sections and preparations to small organisms and live animals.
Several computer workstations and software tools are also made available for advanced image analysis and processing. Regular seminars covering various aspects of light and fluorescence microscopy are offered.
Confocal microscopy with efficient separation of spectrally overlapping fluorophores and optical z-sectioning is beneficial for multi-color 3D fluorescence imaging of many biological structures.

Applications ranging from observing subcellular structures in cells to investigating the morphology of small organisms and large tissue areas at high optical resolution are available (Fig.A/B). Optical clearing of biological samples is often beneficial for imaging of thick specimen by confocal, 2-Photon or light-sheet microscopy (Fig.B). In TIRF microscopy, only fluorophores close to the coverslip will be excited, making this an ideal technique to image dynamic processes close to the cell membrane, e.g. vesicle transport, endocytosis and exocytosis, even providing single molecule sensitivity. 2-Photon (2-P) microscopy is used to image thick, turbid specimen, e.g. tissue preparations, mouse or zebrafish embryos. 2-P systems are also able to measure label-free SHG signals, emitted by highly ordered biological structures, e.g. collagen.
Customised image analysis and modelling based on microscopy-related information reveal how differences in cell motility can induce a directional migration of cardiac tissue in zebrafish heart development (Z. Cseresnyes/AG Seyfried, ref.2).
Photoswitching and -bleaching techniques of structures labelled with genetically-encoded fluorescent proteins allow to monitor the mobility of proteins inside living cells, as well as the tracking of cells in whole live organisms, e.g., to follow morphogenetic processes in zebrafish (A. Sporbert/AG Seyfried, ref.4). Intravital and in vivo imaging of small animals is invaluable for validating results obtained by standard techniques in a physiological environment. The DFG-supported Joint Intravital Microscopy and Imaging platform between R. Niesner, A. Hauser (DRFZ, Berlin) and the Advanced Light Microscopy provides individual, customized support from sample preparation/animal surgery and imaging using standard techniques to intravital microscopy and dedicated image analysis (V. Matiash/Z. Cseresnyes). Supported projects include the imaging of whole mount brain slices (AG Kettenmann) and spleen tissue (AG Höpken).



A) *Tile scan of murine fibrosarcoma tissue: green – tumor cells, red – stromal cells, yellow – endothelial cells, blue – T cells, courtesy of M. Leisegang, AG Uckert*
B) *3D reconstruction of a cleared whole-mount E11.5 mouse embryo, courtesy of H. Schmidt, AG Rathjen*
C) *Quantum dot tri-exciton imaging (QDTI): sub-diffraction resolution imaging of nestin filaments in human neurospheres (ref.1)*
D) *2-Photon imaging of mouse spleen tissue with improved axial resolution by multi-beam striped-illumination (ref.3)*

Method development

Observing subcellular structures is limited by the spatial resolution of light microscopy. A number of new microscopy techniques (termed "superresolution microscopy") have been developed to overcome this limit. However, these techniques require complex microscopy arrangements. We aim at improving the spatial resolution by using available techniques and implementing novel imaging concepts.
In collaboration with M. Heilemann (Goethe University, Frankfurt) we improved a method for 3D sub-diffraction limit imaging based on the ability of quantum dots for three-photon absorption (QDTI), resulting in a smaller point spread function. In combination with deconvolution, up to 1.9-fold improved spatial resolution can be achieved (ref.1). Since QDTI is realized on a confocal microscope it can also be applied to thick, multi-color samples of extended size, i.e. neurospheres (Fig.C). Based on a collaboration with the groups of R. Niesner and A. Hauser (DRFZ, Berlin), a method for high-resolution intravital microscopy is further developed using a multi-beam 2-photon microscope (A. Margineanu/Z. Cseresnyes). This novel approach uses striped-illumination, thus applying the principle of structured illumination and spatial modulation to achieve improved contrast and better axial resolution. This approach was successfully used to visualise cellular interactions in spleen tissue (Fig.D) and in live lymph nodes (ref.3).

Development of a platform management software

A customised management software was developed to make the access and use of the provided infrastructure easy, as well as to ensure a fast communication for support and troubleshooting (5). "ShareMCF" integrates online user- and project databases, registration, microscope booking calendar as well as mailing lists and tools for reporting news and incidents (M. Richter). The MS Sharepoint software can be adapted to the needs of other Core Facilities.

Selected Publications

(1) Simple method for sub-diffraction resolution imaging of cellular structures on standard confocal microscopes by three-photon absorption of quantum dots. **Sporbert A, Cseresnyes Z,** Heidbreder M, Domaing P, Hauser S, Kaltschmidt B, Kaltschmidt C, Heilemann M, Widera D. *PLoS One. 2013* May 21;8(5)

(2) Unilateral dampening of Bmp activity by nodal generates cardiac left-right asymmetry. Veerkamp J, Rudolph F, **Cseresnyes Z,** Priller F, Otten C, Renz M, Schaefer L, Abdelilah-Seyfried S. *Dev Cell. 2013* Mar 25;24(6)

(3) High-resolution intravital microscopy. Andresen V, Pollok K, Rinnenthal JL, Oehme L, Günther R, Spiecker H, Radbruch H, Gerhard J, **Sporbert A, Cseresnyes Z,** Hauser AE, Niesner R. *PLoS One. 2012*;7(12)

(4) Cell tracking using photoconvertible proteins during zebrafish development. Lombardo VA, **Sporbert A,** Abdelilah-Seyfried S. J *Vis Exp. 2012* Sep 28;(67)

(5) A Coustomised web-based tool box for microscopy core facility management. **Cseresnyes Z, Kriegel F, Sporbert A.** *Imaging & Microscopy. 2011,* Issue 4, V

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Photo: Anna Domaska-Akalin

Altuna Akalin

BIMSB Bioinformatics

Research preview

Bioinformatics is the application of computational methods to the management and analysis of biological data. By using bioinformatics, high-dimensional data can be translated to biological knowledge. With the advent of high-throughput technologies in biology, computational analysis needs of the labs are on the rise. This creates a challenge in method development, application and also a demand in training new individuals.

BIMSB Bioinformatics platform creates and maintains bioinformatics tools and databases, and studies biology using those in-house or publicly available computational techniques. In addition, the platform provides collaboration opportunities, helpdesk, and training for MDC scientists. The platform aims to help MDC scientists address their biological questions using and developing computational methods.

On top of these, we have a broad interest in gene regulation, specifically transcriptional regulation and association of transcriptional regulation with epigenomics. We are aiming to use data intensive computational methods to uncover patterns in gene regulation relating to cell differentiation and complex diseases.

DNA modifications and disease

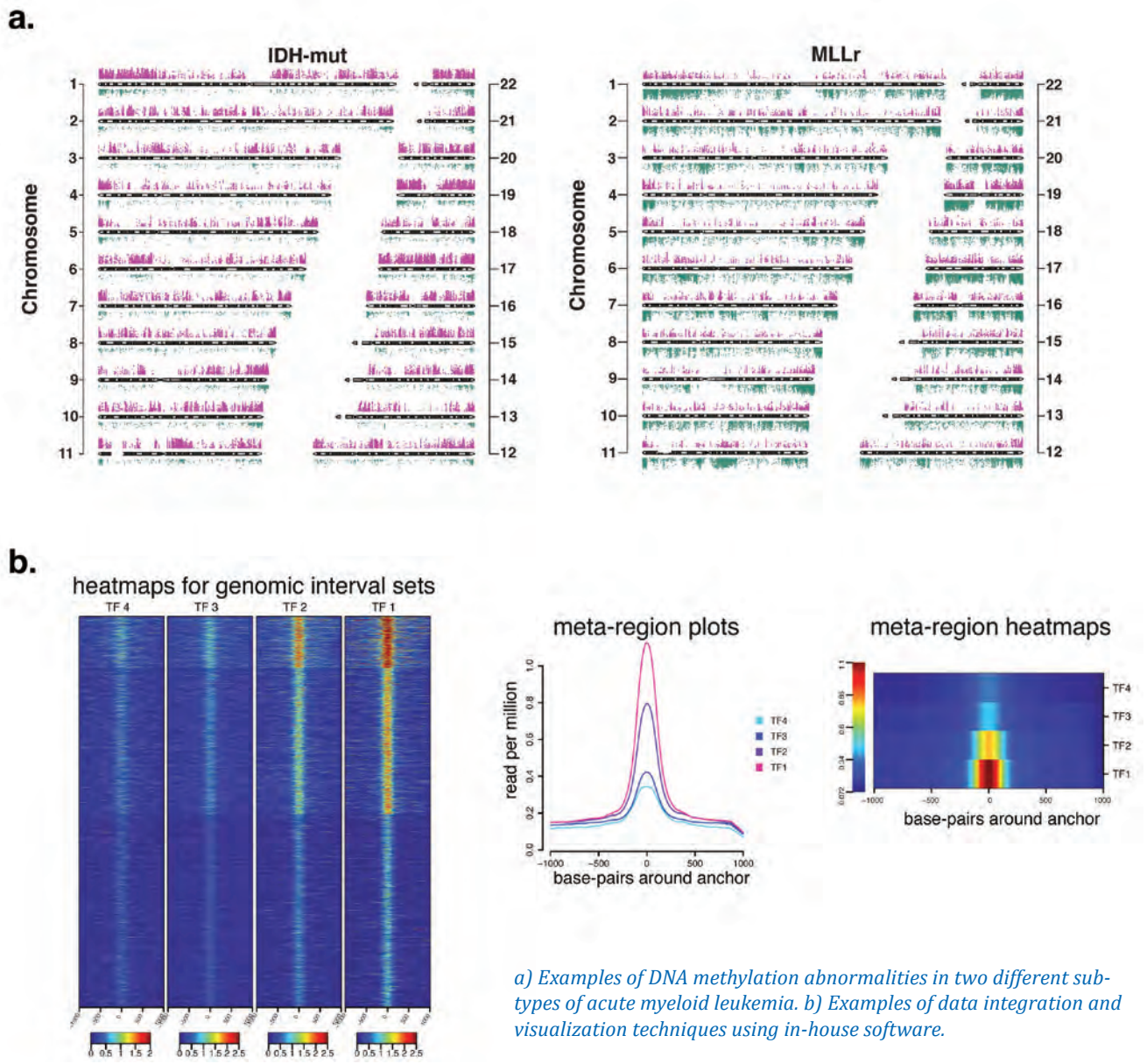
Discovering which abnormalities lead to complex diseases such as cancer remains as a challenge. To complicate the matters, most cases are unique to their selves and do not allow for simple all-embracing modeling of the disease. However, our previous work has shown that certain types of cancers share mutations on pathways relating to DNA modifications, such as methylation and hydroxymethylation. Such modifications are previously shown to be associated with gene regulation. These new class of cancers are distinguished by certain abnormalities in their epigenomic profiles, which paves way for different approaches in patient stratification and drug design. We analyze high-throughput sequencing data sets to investigate epigenomic abnormalities and differences in various normal and disease cell types.

Computational analysis of multi-level gene regulation

Gene regulation is a complex event where multiple partners interact with each other to achieve gene regulation. The level of expression of a gene is initially controlled by transcriptional regulatory mechanisms. This is followed by post-transcriptional mechanisms, which can further modify transcript levels. In order to have full picture on gene-expression, one must take into account all the different players in these processes. The lab will aim to work on multi-level gene regulation by utilizing bioinformatics tools with high-throughput data sets such as CAGE, RNA-seq, ChIP-seq and PAR-CLIP.

Data-integration and machine learning approaches

Most successful approaches in modeling complex phenomena, such as complex



a) Examples of DNA methylation abnormalities in two different subtypes of acute myeloid leukemia. b) Examples of data integration and visualization techniques using in-house software.

diseases or gene regulation, make use of different types of data. These data sets should not only be limited to high-throughput sequencing based data sets mentioned above, but also should include proteomics & metabolomics data sets. We believe only through integration of multi-layered data, complex biological processes can be understood in a system-wide level. This requires efficient preprocessing, integration, analysis and visualization of diverse data sets.

In principal, integration and analysis problem of big data sets can be solved by translating computer science and statistics research to biology, and developing those methods further where necessary. In collaboration with other platforms and groups at BIMSB and MDC, we are aiming to use statistics and machine-learning techniques to uncover relationships in high-dimensional biological data from diverse sources.

Selected Publications

Lu C, Venneti S, **Akalin A**, Fang F, Ward PS, Dematteo RG, Intlekofer AM, Chen C et al. Induction of sarcomas by mutant IDH2. Genes Dev. 2013 Sep 15;27(18):1986-98

Akalin A*, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, Mason CE. methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. Genome Biology, 2012 Oct 3;13(10):R87* (Co-corresponding author)

Akalin A, Garrett-BakelmanFE, KormakssonM, BusuttilJ, et al. Base-pair resolution DNA methylation sequencing reveals profoundly divergent epigenetic landscapes in acute myeloid leukemia.. PLoS Genetics 2012;8(6):e1002781.

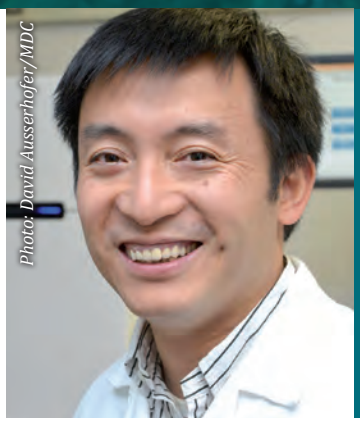
PlessyC*,Pascarella G*, Bertin N*, **Akalin A*,** CarrieriC, VassalliA, LazarevicD, et al. Promoter architecture of mouse olfactory receptor genes. Genome Research. 2011 Dec 22. * (Equal Contribution)

Akalin A, Fredman D, Arner E, Dong X, Bryne JC, Suzuki H, Daub CO, Hayashizaki Y, Lenhard B. Transcriptional features of genomic regulatory blocks. Genome Biol. 2009;10(4):R38

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Photo: David Ausserhofer/MDC

Wei Chen

BIMSB Genomics

The recent introduction of novel sequencing technology (NGS) has revolutionized genomic research. As one of the leading NGS facilities in Europe, our lab has been providing NGS supports for both the researchers within MDC and external collaborators. The services ranges from whole genome (re)sequencing, targeted genome sequencing, transcriptome sequencing to various epigenomic profilings. Apart from offering 'just' the services to the institute as a core facility, my lab has been developing various NGS-based genomic assays (see BIMSB).

NGS instrument and applications

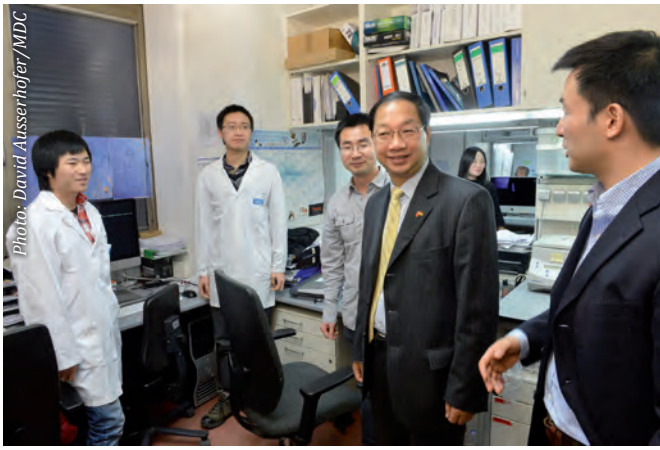
BIMSB genomics platform is now equipped with most of the currently available next generation sequencing systems, including one 454 FLX (Roche), two SOLiD 4 (life technologies), one GAIIX, two HiSeq2000 and two Hiseq 2500 (Illumina), and one PacBio SMRT sequencing (PacBio). With these resources, a totle of 4,313 samples have been sequenced between 2011-1-1 and 2013-12-31. The raw sequencing data amounts to >52 Terabases. Our current NGS applications include:

Exome sequencing

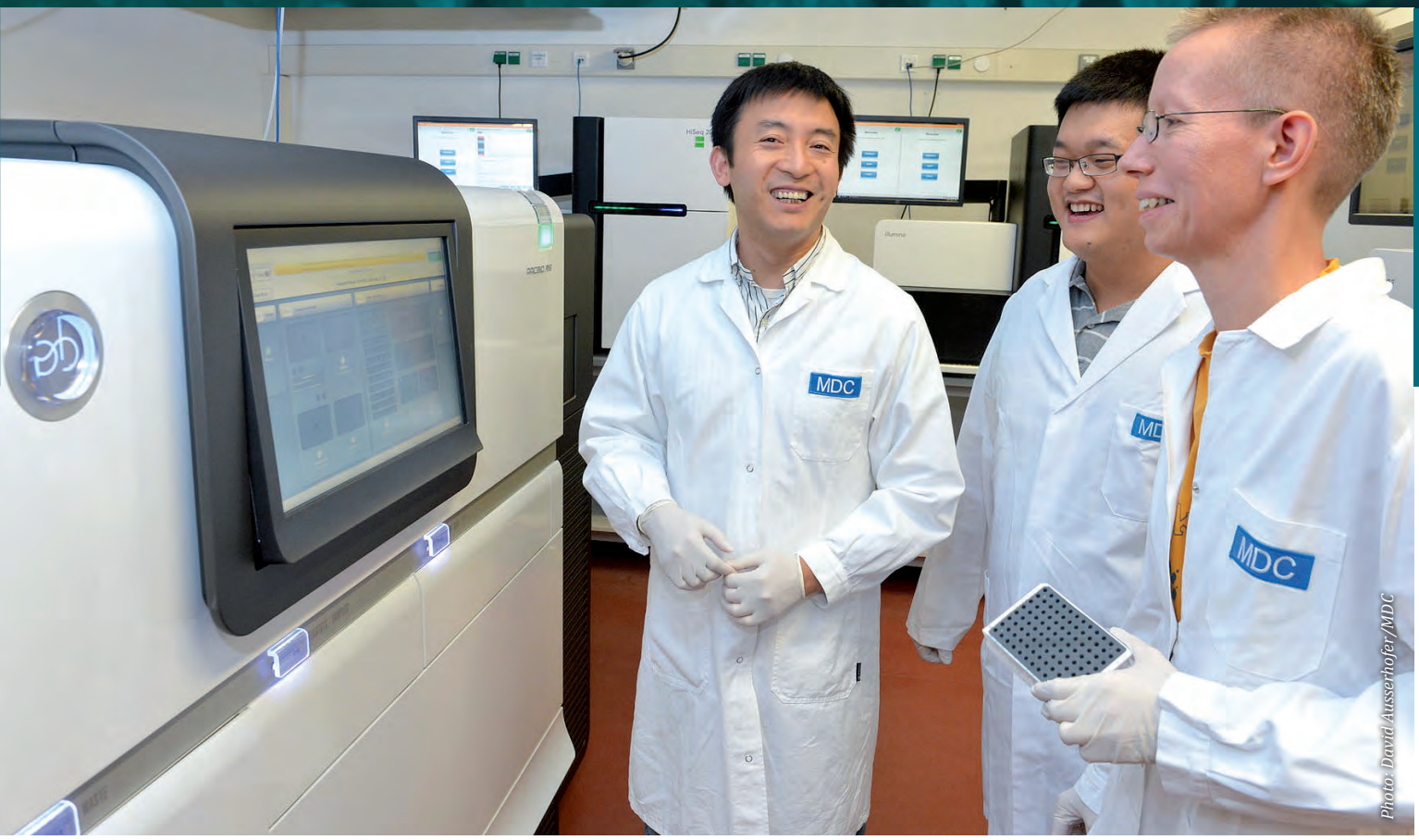
The Agilent SureSelect V5 exome enrichment system is used to prepare exome sequencing libraries. With a sequencing output of ~10Gb, an average of ~80% of all reads fall within the targeted regions +/- 100bp and ~95% of all targeted bases are covered >= 20x. A sequencing output of ~10Gb per exome sample can be achieved on a thoroughly optimized workflow using the HiSeq2000 system and the SureSelect V5 enrichment system, generating ~200 million single end 100nt reads for each probe of a 2-plex pool on one lane.

Whole-genome sequencing

The Illumina Nextera whole-genome library preparation kit features the rapid creation of gDNA libraries from very low input sample concentration (> 50ng genomic DNA). During library creation, a process termed 'tagmentation' simultaneously fragments and tags DNA, making fragments amenable to direct PCR amplification. A sequencing output of ~80Gb per genome sample, which is required to cover the whole genome with >20x, can be achieved on a thoroughly optimized workflow using 2 lanes on the HiSeq2000 system, generating ~200 million paired-end 2x100nt reads on one lane.


Photo: David Ausserhofer/MDC

Visit of H.E.Shi Mingde (2nd from right), Ambassador of the People's Republic of China.



Wei Chen (left) with the PacBio RS System sequencer in his lab.

RNA-seq

Long RNA samples are processed in content-defined batches, standardized paired-end libraries will be created using established protocols (modified Truseq Chemistry). up to three samples can be labeled with different barcodes and pooled for sequencing of 100 nt from single end in one lane, with a minimum of 50 million reads for each sample.

Small RNA-seq

Starting from 1µg of total RNA, a size selection is performed for RNAs between 18 and 30 nt to enrich for small RNAs, including miRNAs. Subsequent library preparation steps include: adapter ligation and PCR amplification. The final smallRNA library is obtained by a further size selection step. Up to 12 samples can be labelled with unique barcodes and pooled for sequencing, 1x50nt single-end, with a minimum of 10 million reads per sample.

ChIP-seq

Starting from 10-20ng of ChIP-DNA, the following library preparation steps are performed: end-repair, a-tailing, adapter ligation, gel purification and a final PCR enrichment. After quality control of the sequencing library, up to three sample can be labeled with unique barcodes and pooled for sequencing, 1x50nt single-end, in one lane with a minimum output of 50 million reads for each sample.

DNA methylation analysis

Methylome–wide analysis is currently performed using Reduced Representation Bisulfite Sequencing (RRBS). The RRBS-method was selected because DNA methylation pattern can be analyzed on the nucleotide level in CpG island and CpGI shores with a coverage of around 10 % of all CpG positions. Moreover this method is feasible with a very low amount of DNA. Up to three RRBS samples can be labeled with different barcodes and pooled for sequencing of 100 nt from single end in one lane, with a minimum of 50 million reads for each sample.

Group Leader
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Complete list of team members can be found in chapter Berlin Institute for Medical Systems Biology, Group “Novel sequencing technology, Functional and medical genomics”



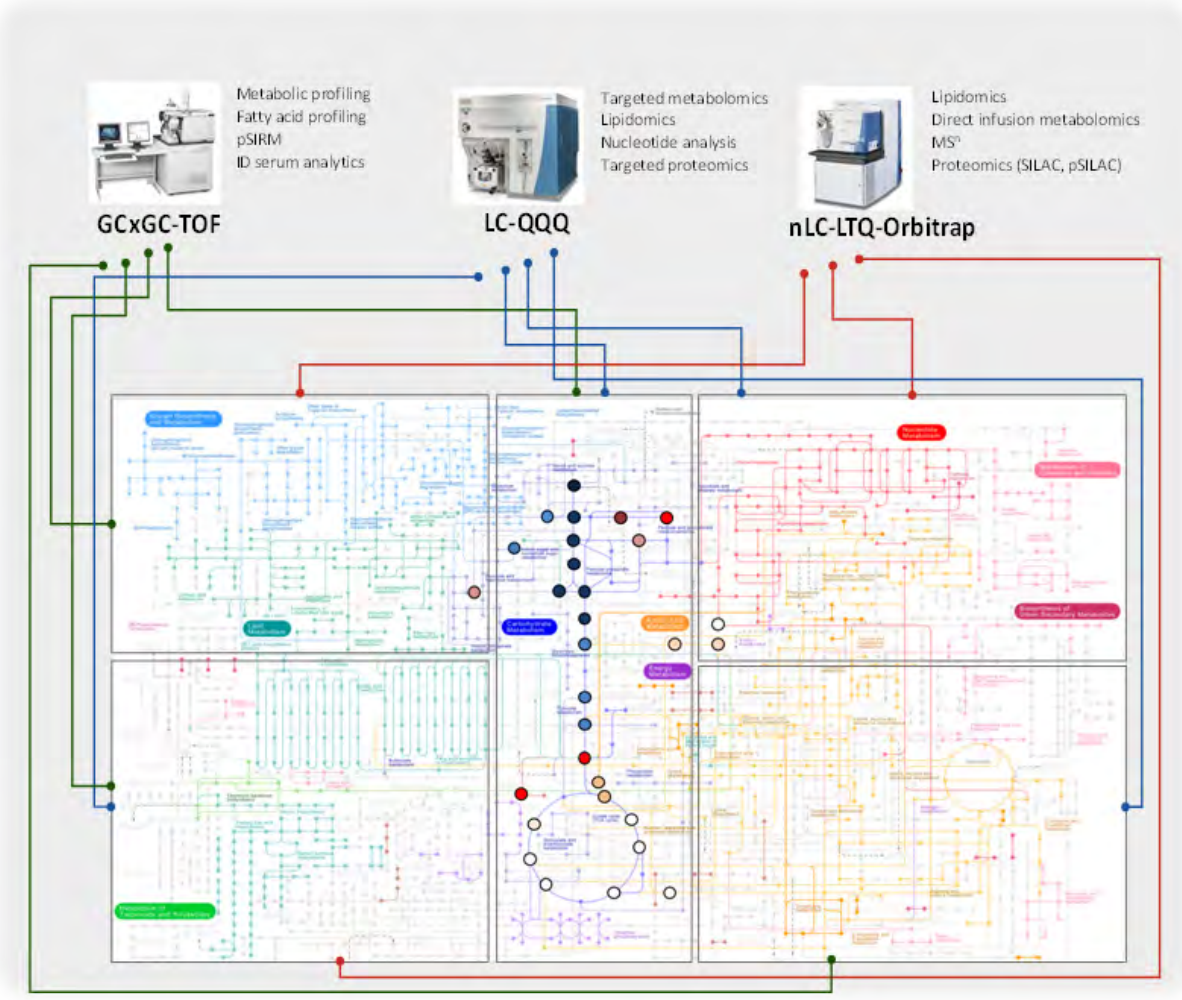
Photo: David Ausserhofer/MDC

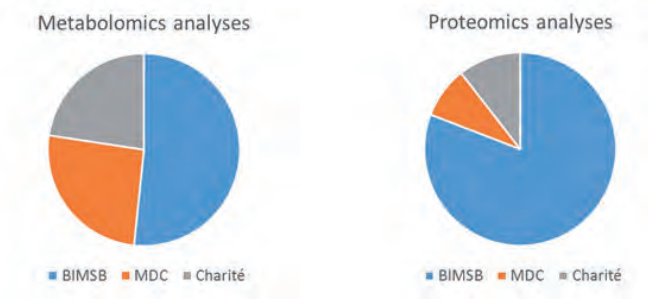
Stefan Kempa

BIMSB Proteomics & Metabolomics

Overview about the mass spectrometers installed at BIMSB and related methods. During the last years we have installed a GC-ToF-MS, a triple-quadrupole-MS and a LTQ-Orbitrap-Velos. The scheme shows the mass spectrometers, established methods and related metabolic pathways that can be analyzed.

Within the past decades biochemical data of single processes, metabolic and signaling pathways were collected and advances in technology led to improvements of sensitivity and resolution of bioanalytical techniques. These achievements build the bases for the so called 'genome wide biochemistry'.





Overview about the metabolomics and proteomics analyses performed by the group. Visualized are the analyses performed within projects related to the associated institutions. In total 18,000 metabolomics and 4,500 proteomics samples were processed.

Single level study of a living organism cannot give a complete understanding of the mechanism regulating biological functions. The integration of transcriptomics, proteomics and metabolomics data with existing knowledge allows connecting biological processes which were treated as independent so far. In this context the aim of our group is to establish and apply metabolomics and proteomics techniques for absolute quantification and to analyze turnover rates of proteins and metabolites using stable isotopes.

In order to decode molecular mechanisms causing metabolic disorders (a) a dynamic view on metabolism and (b) information about the expression levels of proteins, eg. metabolic enzymes and regulatory proteins, is required. Using the established techniques we are able to measure the metabolic activity of cells, organs and organisms in a dynamic manner in vitro and in vivo. We support BIMSB and MDC groups in frame of scientific collaborations to address complex biological questions by applying cutting edge metabolomics and proteomics techniques:

- Metabolic profiling (targeted, non-targeted, lipid profiling)
- Stable isotope resolved metabolomics (in cell cultures and *in vivo*)
- Proteome analyses (SILAC, *in vivo* SILAC and targeted proteomics)

At present the integrative proteomics and metabolomics platform at BIMSB supports the setup of the BIH metabolomics core by implementing the technologies and workflows that were established at BIMSB during the last years.

Selected Publications

Synthetic lethal metabolic targeting of cellular senescence in cancer therapy. Doerr, J.R. and Yu, Y. and Milanovic, M. and Beuster, G. and Zasada, C. and Daebritz, J.H.M. and Lisec, J. and Lenze, D. and Gerhardt, A. and Schleicher, K. and Kratzat, S. and Purfuerst, B. and Walenta, S. and Mueller-Klieser, W. and Graeler, M. and Hummel, M. and Keller, U. and Buck, A.K. and Doerken, B. and Willmitzer, L. and Reimann, M. and Kempa, S. and Lee, S. and Schmitt, C.A. Nature 501 : 421-425. 19 September 2013

Deregulated MYC expression induces dependence upon AMPK-related kinase 5. Liu, L. and Ulbrich, J. and Mueller, J. and Wuestefeld, T. and Aeberhard, L. and Kress, T.R. and Muthalagu, N. and Rycak, L. and Rudalska, R. and Moll, R. and Kempa, S*. and Zender, L. and Eilers, M. and Murphy, D.J. Nature 483 (7391): 608-612. 29 March 2012 (corr. for metabolomics/proteomics)

Proteome dynamics and early salt stress response of the photosynthetic organism Chlamydomonas reinhardtii. Mastrobuoni, G. and Irgang, S. and Pietzke, M. and Wenzel, M. and Assmus, H.E. and Schulze, W.X. and Kempa, S. BMC Genomics 13 (1): 215. 31 May 2012

De novo assembly and validation of planaria transcriptome by massive parallel sequencing and shotgun proteomics. Adamidi, C. and Wang, Y. and Gruen, D. and Mastrobuoni, G. and You, X. and Tolle, D. and Dodt, M. and Mackowiak, S.D. and Gogol-Doering, A. and Oenal, P. and Rybak, A. and Ross, E. and Alvarado, A.S. and Kempa, S*. and Dieterich, C. and Rajewsky, N. and Chen, W. Genome Research 21 (7): 1193-1200. July 2011 (*co-corresponding author)

Muscle-type creatine kinase interacts with central domains of the M-band proteins myomesin and M-protein. Hornemann, T. and Kempa, S. and Himmel, M. and Hayess, K. and Fuerst, D.O. and Wallimann, T. Journal of Molecular Biology 332 (4): 877-887. 26 September 2003

Group Leader
Dr. Stefan Kempa

Complete list of team members can be found in chapter Berlin Institute for Medical Systems

Biology, Group "Metabolic Regulation"



Photo: David Ausserhofer/MDC

Bettina Purfürst

Electron Microscopy

The EM facility supports all research groups of the MDC with expertise and technology to study biological probes like macromolecules, isolated cell organells, cells, tissue and clinical samples. In the period reported, a new 11 Megapixel high speed CCD camera was installed on the Zeiss EM 910, resulting in a much faster generation of large picture series for phenotyping and quantification. Here are some selected topics from the last two years:

Quantification of subcellular compartments

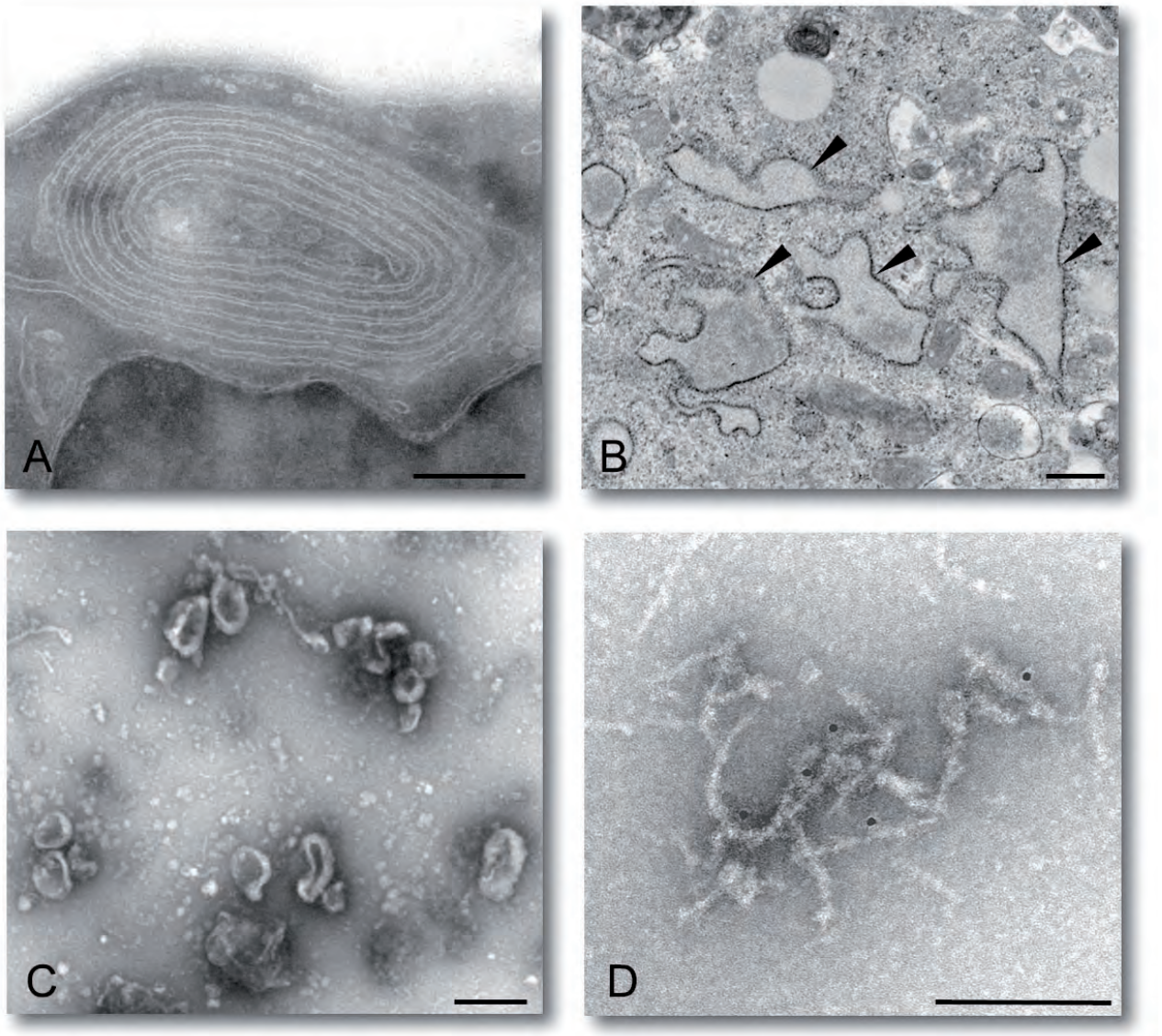
In two cases, we could detect and even quantify endoplasmatic reticulum (ER) stress in different model systems in cancer research. High-grade astrocytoma cells often contain a proliferated, parallel ER under normal cultivation conditions (Figure A). Vanilloid receptor agonists released by neural precursor cells induced a strong dilation of the ER lumen and finally the death of these astrocytoma cells (Stock et al., 2012). A similar proliferated ER with large cisternae could be quantified in a mouse lymphoma cell system (Figure B), where the ER stress was closely related to autophagy and senescence (Dörr et al., 2013). In further projects, we quantified the number and area of mitochondria in smooth muscle cells and the density of insulin granula in beta cells of the pancreas (collaborations with F. Le Noble and C. Birchmeier).

Negative contrast

This method allows a direct visualization of purified proteins and other macromolecules at high resolution. Here, we detected for the first time a nearly homogeneous population of 150 – 200 nm vesicles released from human myotubes (Figure C, Zacharias et al., 2011), originating from patients with muscular dystrophies. Similar membrane microvesicle fractions, called exosomes, are secreted by various cell types and may have a promising diagnostic and therapeutic potential. In another approach, we successfully applied immunolabeling to negative contrast probes, as shown for amyloid fibrils in Figure D (collaboration with A. Böddrich).

Structure of heart and skeletal muscle under normal and pathological conditions

We have characterized a large range of heart and skeletal muscle probes for ultrastructural changes in different phenotypes (collaborations with L. Thierfelder, M. Gotthard and B. Schröder from the MDC, S. Spuler and J. Fielitz from the Charite and ECRC). Normal features of this type of tissue are easy to quantify, like sarcomere length, desmosome structure, and shape, number and inner membrane structure of mitochondria, followed by secondary effects like an increased amount of lipid droplets. In strong phenotypes, the Z-line structure is heavily disturbed (minicore disease) and may result in a completely disintegrated, amorphous sarcomere. In some cases, we successfully immunolabeled proteins in cell-cell contacts (Desmoplakin, Plakophilin 2 and Desmin) and in Ca^{2+} channels of the heart (collaborations with A. Heuser and H. Haase).



A: *Endoplasmatic reticulum (ER) in high-grade astrocytoma cells grown in control medium. The ER is proliferated and arranged in parallel stacks, but the ER lumen remains thin, see Stock et al., 2012.Cryosection according to Tokuyasu, membranes appear white, bar = 500 nm*
B: *ER in lymphoma cells from a mouse model system, treated with adriamycin. The ER is proliferated and shows highly dilated cisternae (arrowheads), see Dörr et al., 2013.Epon-embedding, membranes appear dark, with black ribosomes attached, bar = 500 nm*
C: *A population of microvesicles released from human myotubes, see Zacharias et al., 2011.Negative contrast, bar = 200 nm*
D: *Amyloid fibrils, immunolabeled with 12 nm colloidal gold particles.Negative contrast, bar = 200 nm*

Selected Publications

U Zacharias, B Purfürst, V Schöwel, I Morano, S Spuler, and H Haase. Ahnak1 abnormally localizes in muscular dystrophies and contributes to muscle vesicle release.J Muscle Res Cell Motil 32:271-280 (2011)

K Stock, J Kumar, M Synowitz, S Petrosino, R Imperatore, ESJ Smith, P Wend, B Purfürst, UA Nuber, U Gurok, V Matyash, J-H Wälzlein, SR Chirasani, G Dittmar, BF Cravatt, S Momma, GR Lewin, A Ligresti, L De Petrocellis, L Cristino, V Di Marzo, H Kettenmann, R Glass. Neural precursor cells induce cell death of high-grade astrocytomas through stimulation of TRPV1.Nature Medicine 18: 1232-1238 (2012)

D Bröhl, E Vasyutina, MT Czajkowski, J Griger, C Rassek, H-P Rahn, B Purfürst, H Wende, C Birchmeier. Colonization of the satellite cell niche by skeletal muscle progenitor cells depends on notch signals.Developmental Cell 23: 469-481 (2012)

H Waiczies, S Lepore, S Drechsler, F Qadri, B Purfürst, K Sydow, M Dathe, A Kühne, T Lindel, W Hoffmann, A Pohlmann, T Niendorf, S Waiczies. Visualizing brain inflammation with a shingled-leg radio-frequency head probe for $^{19}F/^{1}H$ MRI.Scientific Reports 3: 1280 (2013)

JR Dörr, Y Yu, M Milanovic, G Beuster, C Zasada, JHM Däbritz, M Reimann, J Lisec, K Schleicher, S Kratzat, B Purfürst, S Walenta, W Müller-Klieser, U Keller, AK Buck, B Dörken, L Willmitzer, S Kempa, S Lee, CA Schmitt. Synthetic Lethal Metabolic Targeting of Cellular Senescence in Cancer Therapy.Nature 501: 421-25 (2013)

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Photo: Peter Sonnabend/NGFN

Erich Wanker

Interactomics

Interactome

The relevance of interactions between proteins for biological inquiry is beyond dispute. Several methods to identify protein-protein interactions (PPIs) are well-established. One method that has been developed into an automated high-throughput system is the yeast-two-hybrid method. It allows screening of binary protein-protein interactions and compilation of large interaction network maps. These networks are the raw material for many disciplines: Experimental biologists select subsets of interactions to form and test new hypotheses on protein function and molecular mechanisms, computational biologists use them to predict biologically relevant interactions, systems biologists derive an understanding of how the proteome-wide interactome is organised.

Background

The research group of Prof. Erich Wanker is one of the pioneering labs in the field. We have put forward the first PPI map of a human disease, Huntington's disease (Goehler et al., 2004, Molecular Cell) as well as the first proteome-wide interactome map for human (Stelzl et al., 2005, Cell). In the course of these studies we have established a platform of automated screening and validation techniques which we offer for use to all scientists at the MDC and outside.

Platform technologies

The automated yeast-two-hybrid system. It was first described by Fields and Song (1989, Nature) and is based on the principle of entering bait and prey plasmids of interest in a genetically modified yeast strain. If the protein products interact, a reporter gene is activated that enables the yeast's growth on selective medium. A colour reaction makes clones with interacting proteins visible. The system is particularly suited to detecting also low-affinity or transient interactions. This makes it prone to identifying also false positives, which is why we have established comprehensive quality scoring and validation techniques.

The membrane yeast-two-hybrid system. MYTH is a split-ubiquitin based system to detect interactions between integral membrane proteins and membrane-associated/proximal proteins (Stagljar et al., 1998, PNAS).

The cytosolic yeast-two-hybrid system. CytoY2H is a split-ubiquitin-based cytosolic screening system to detect interactions between transcriptionally active proteins and enables the detection of PPIs in the cytoplasm (Mockli et al., 2007, Biotechniques).

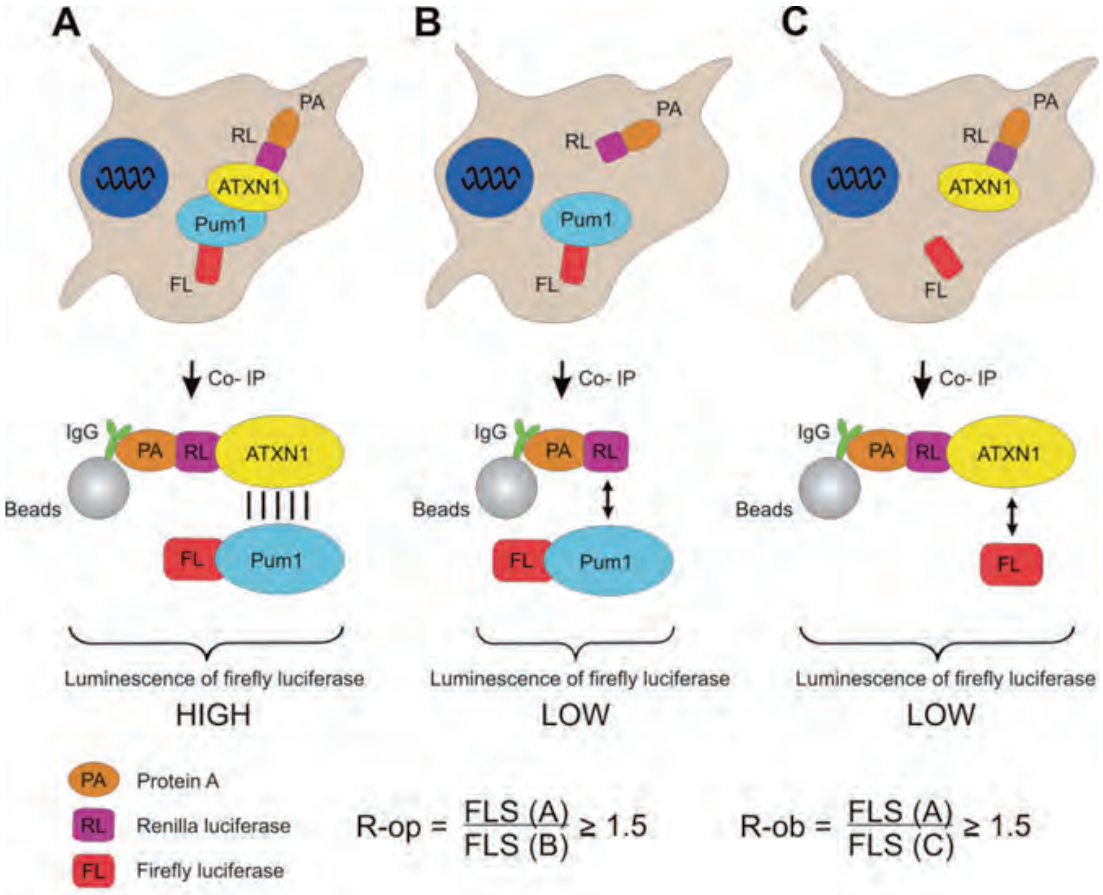
The LUMIER assay. This is a luminescence-based mammalian interactome screening technology (Barrios-Rodiles et al., Science, 2005) for the identification of PPIs in mammalian cells, which we have established for validation of PPIs detected in the Y2H system. Like the Y2H techniques, it has been automated and can be applied to high-throughput testing of interactions.

The clone library. For the interaction screenings, a comprehensive prey-library arrayed in microtiter plates is available. It contains ~23,000 entry-clones, of which ~14,000 are unique full-length cDNAs. This library covers more than 75% of the human genome. We are able to re-array the library according to the needs and interests of diverse research projects.

Identification of Human Proteins that Modify Misfolding and Proteotoxicity of Pathogenic Ataxin-1

Petrakis et al., 2012, PLoS Genetics

In this study, we sought to identify proteins that interact with Ataxin-1, causative to spinocerebellar ataxia type 1, a rare neu-



Schematic representation of a cell-based LUMIER co-immunoprecipitation assay. *To investigate the interaction between the proteins ATXN1Q30 (bait) and Pum1 (prey) the fusion protein pairs (A) PA-RL-ATXN1Q30/FL-Pum1, (B) PA-RL/FL-Pum1 and (C) PA-RL-ATXN1Q30/FL were co-produced in HEK293 cells. After 48 h the cells were lysed and protein extracts were applied to IgG coated magnetic beads (Dynabeads; Invitrogen) for co-immunoprecipitation (co-IP) of interacting proteins. Following the co-IP the firefly luminescence signal was determined for all three samples (A–C) using a luminescence plate reader. In sample A, as a result of the interaction between PA-RL-ATXN1Q30 and FL-Pum1 a high luminescence signal was detected. In comparison, relatively low signals were obtained in control samples B and C. Using the measured values we calculated the binding ratios R-op and R-ob, which are a measure for the specificity of the ATXN1Q30 and Pum1 interaction. Based on previous empirical studies with well-characterized interaction test pairs, we define an interaction as positive when the calculated R-op and R-ob ratios are >1.5.*

rodegenerative disease that belongs to the polyglutamine repeat disorders. We investigated a selection of interactions with Ataxin-1 in our LUMIER assay (Figure 1), identifying PUM1 as an interactor that promotes misfolding and aggregation of the Ataxin-1 protein. Using a PPI screening and validation strategy, biologically relevant proteins and pathways can be identified.

Selected Publications

H. Goehler, M. Lalowski, U. Stelzl, S. Waelter, M. Stroedicke, U. Worm, A. Droege, K. S. Lindenberg, M. Knoblich, C. Haenig, M. Herbst, J. Suopanki, E. Scherzinger, C. Abraham, B. Bauer, R. Hasenbank, A. Fritzsche, A. H. Ludewig, K. Büssow, S. H. Coleman, C.-A. Gutekunst, B. G. Landwehrmeyer, H. Lehrach, and E. E. Wanker, "A protein interaction network links GIT1, an enhancer of huntingtin aggregation, to Huntington's disease.," Mol. Cell, vol. 15, no. 6, pp. 853–865, 2004.

U. Stelzl, U. Worm, M. Lalowski, C. Haenig, F. H. Brembeck, H. Goehler, M. Stroedicke, M. Zenkner, A. Schoenherr, S. Koeppen, J. Timm, S. Mintzlaff, C. Abraham, N. Bock, S. Kietzmann, A. Goedde, E. Toksöz, A. Droege, S. Krobitsch, B. Korn, W. Birchmeier, H. Lehrach, and E. E. Wanker, "A human protein-protein interaction network: a resource for annotating the proteome.," Cell, vol. 122, no. 6, pp. 957–968, 2005.

K. Venkatesan, J.-F. Rual, A. Vazquez, U. Stelzl, I. Lemmens, T. Hirozane-Kishikawa, T. Hao, M. Zenkner, X. Xin, K.-I. Goh, M. A. Yildirim, N. Simonis, K. Heinzmann, F. Gebreab, J. M. Sahalie, S. Cevik, C. Simon, A.-S. de Smet, E. Dann, A. Smolyar, A. Vinayagam, H. Yu, D. Szeto, H. Borick, A. Dricot, N. Klitgord, R. R. Murray, C. Lin, M. Lalowski, J. Timm, K. Rau, C. Boone, P. Braun, M. E. Cusick, F. P. Roth, D. E. Hill, J. Tavernier, E. E. Wanker, A.-L. Barabási, and M. Vidal, "An empirical framework for binary interactome mapping.," Nat. Methods, vol. 6, no. 1, pp. 83–90, 2009.

A. Vinayagam, U. Stelzl, R. Foulle, S. Plassmann, M. Zenkner, J. Timm, H. E. Assmus, M. A. Andrade-Navarro, and E. E. Wanker, "A directed protein interaction network for investigating intracellular signal transduction.," Sci. Signal., vol. 4, no. 189, p. rs8, 2011.

S. Petrakis, T. Raskó, J. Russ, R. P. Friedrich, M. Stroedicke, S.-P. Riechers, K. Muehlenberg, A. Möller, A. Reinhardt, A. Vinayagam, M. H. Schaefer, M. Boutros, H. Tricoire, M. A. Andrade-Navarro, and E. E. Wanker, "Identification of human proteins that modify misfolding and proteotoxicity of pathogenic ataxin-1.," PLoS Genet., vol. 8, no. 8, p. e1002897, Jan. 2012.

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Susanne Köppen



Photo: David Ausserhofer/MDC

Thoralf Niendorf

Magnetic Resonance (Berlin Ultrahigh Field Facility, B.U.F.F.)

B.U.F.F. has been implemented on the Campus Berlin-Buch. B.U.F.F. builds on collaborations with our strategic partners from the Charité, the Leibniz Institute for Molecular Pharmacology (FMP), the Physikalisch-Technische Bundesanstalt (PTB), Siemens Healthcare, Bruker Biospin and other (inter)national institutions. To learn more about the mission of B.U.F.F. please visit
https://www.mdc-berlin.de/37752989/en/ecrc/Ultrahigh_Field_MR/B_U_F_F_

Equipment and infrastructure

B.U.F.F. provides state-of-the-art infrastructure for interdisciplinary imaging projects and is equipped with small animal (9.4 T) and whole-body human (7.0 T & 3.0 T) MR scanners **(Figure 1)**. For experimental and preclinical imaging B.U.F.F. runs an S1 certified laboratory and supports small scale animal housing. To foster translational research and explorations into clinical studies B.U.F.F. is equipped with 2 reception areas to comfort and guide volunteers and patients. B.U.F.F. comprises four dressing rooms, all connected to scanner rooms and reception areas **(Figure 2, left)**. Several web interface based reading stations are available; imaging data can be accessed from every desktop available at B.U.F.F. All technical prerequisites needed for clinical studies are setup including emergency equipment, extra patient monitoring units, defibrillators and extra MR safe patient beds. Offices used for research staff are separated from the scanner areas to meet the needs for data safety and volunteer/patient convenience. To meet all data safety requirements B.U.F.F. is equipped with a commercial PACS system used for data storage, data archiving and data mining. The PACS system is connected with our data base BUFFportal, which is used for safety training, comprehensive project management, data handling, publication tracking, etc. To push the capabilities of magnetic resonance and to drive next generation MR methodology and MR hardware B.U.F.F. provides infrastructure for radiofrequency (RF) coil design, for MR pulse sequence development, for development, validation and application of ancillary hardware and for rapid prototyping **(Figure 2, right)**.

Research focus

Research at B.U.F.F. concentrates on the development of MR methodology and MR technology with a focus on new ways of mapping and probing anatomy, morphology, microstructure, function, physiology and metabolism together with explorations into the benefits and challenges of ultrahigh field (UHF) imaging. These efforts are designed to spatially resolve and characterize (patho)physiological and biophysical processes to promote a transfer from basic research to (pre)clinical studies and vice versa. To highlight the anatomical detail and spatial resolution capabilities of MRI **Figure 3** surveys examples derived from in vivo mice phenotyping and from in vivo human imaging.

Contributions to major research initiatives

Principle investigators of B.U.F.F. form a key/integral part of major initiatives including the Helmholtz Alliance for Imaging and Curing Environmental Diseases (ICEMED), the population MRI program of the German National Cohort (GNC), the DFG research group FOR 1368 on Hemodynamics of Acute Kidney Injury, the imaging program of the German Center for Cardiovascular Research (DZHK), the EU funded FP7 project INSERT on hybrid SPECT/MR imaging, the BMBF initiative on sodium MRI (NAMRIS) and the German Ul-



Fig. 1: *Picture photographs of the whole-body human (7.0 T & 3.0 T) and small animal (9.4 T) MR scanner environments at B.U.F.F.*

Fig. 2: *Overview of the volunteer/patient preparation areas, the clinical imaging setup and the MR hardware development laboratories available at B.U.F.F.*

trahigh Field Imaging (GUFI) network. B.U.F.F. is an integral part of the imaging core facility of the Berlin Institute of Health (BIH).

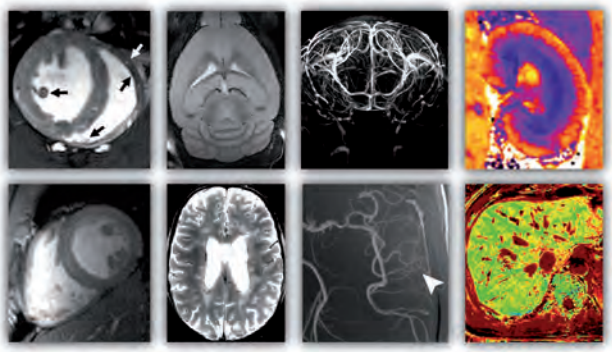
How to get to there

B.U.F.F. is easily accessible via public transportation as well as by car. Go green and use public transport. Public transportation is directly arriving/leaving on campus Berlin-Buch (line 353 from S-Bahn station Berlin-Buch to station Lindenberger Weg-Süd). The bus station is located right in front of B.U.F.F. Free parking space is available in front of B.U.F.F.

Basic research and clinical science opportunities

The B.U.F.F. team is always looking for collaborators. For further details about opportunities for collaboration, access, resources and projects please visit *https://buffportal.mdc-berlin.de* or *www.mdc-berlin.de/BUFF*.
The user guidelines can be found under *https://buffportal.mdc-berlin.de./procedure*.
For further details please contact the Head of B.U.F.F. (Thoralf Niendorf, *thoralf.niendorf@mdc-berlin.de*, +49 30 9406 4505).

Fig. 3:*Illustration of the anatomical detail and spatial resolution provided by MR imaging.* **Top:** *In vivo MR images of the mice heart (in-plane spatial resolution 50 micrometer), of the mice brain (in-plane spatial resolution 30 micrometer), of the vessel tree of the mice brain (in-plane spatial resolution 30 micrometer) and of the mice kidney (in-plane spatial resolution 150 micrometer) obtained with the 9.4 T small animal MR scanner at B.U.F.F.* **Bottom:** *In vivo MR images of the human heart (in-plane spatial resolution 1.0 mm), of the human brain (in-plane spatial resolution 0.5 mm), of the left hemisphere vessel tree of the human brain (in-plane spatial resolution 0.5 mm) and of the human liver (in-plane spatial resolution 1.0 mm) obtained with the 7.0 T whole body human MR scanner at B.U.F.F.*



Patents / Patent applications

please see the chapter on Ultrahigh Field Magnetic Resonance (UHF-MR)

Selected Publications

Pohlmann A, Cantow K, Hentschel J, Arakelyan K, Ladwig M, Flemming B, Hoff U, Persson PB, Seeliger E, Niendorf T. Linking non-invasive parametric MRI with invasive physiological measurements (MR-PHYSIOL): towards a hybrid and integrated approach for investigation of acute kidney injury in rats. Acta Physiol (Oxf) 2013;207(4):673-689.

Sinnecker T, Dorr J, Pfueller CF, Harms L, Ruprecht K, Jarius S, Bruck W, Niendorf T, Wuerfel J, Paul F. Distinct lesion morphology at 7-T MRI differentiates neuromyelitis optica from multiple sclerosis. Neurology 2012;79(7):708-714.

Kopp C, Linz P, Wachsmuth L, Dahlmann A, Horbach T, Schofl C, Renz W, Santoro D, Niendorf T, Muller DN, Neininger M, Cavallaro A, Eckardt KU, Schmieder RE, Luft FC, Uder M, Titze J. (23)Na magnetic resonance imaging of tissue sodium. Hypertension 2012;59(1):167-172.

von Knobelsdorff-Brenkenhoff F, Tkachenko V, Winter L, Rieger J, Thalhammer C, Hezel F, Graessl A, Dieringer MA, Niendorf T, Schulz-Menger J. Assessment of the right ventricle with cardiovascular magnetic resonance at 7 Tesla. J Cardiovasc Magn Reson 2013;15:23.

Ku MC, Wolf SA, Respondek D, Matyash V, Pohlmann A, Waiczies S, Waiczies H, Niendorf T, Synowitz M, Glass R, Kettenmann H. GDNF mediates glioblastoma-induced microglia attraction but not astrogliosis. Acta Neuropathol 2013;125(4):609-620.

Structure of the Group

please see the chapter on Ultrahigh Field Magnetic Resonance (UHF-MR)



Photo: David Ausserhofer/MDC

Gunnar Dittmar

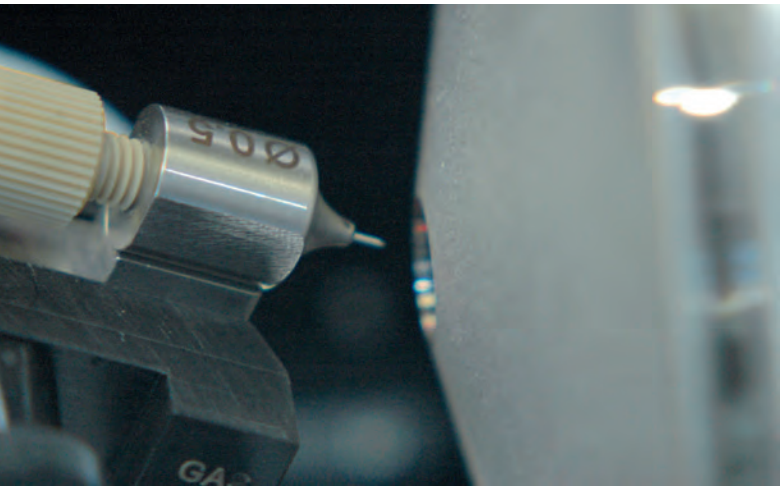
Mass Spectrometry

Cellular signaling

Cells interact with their environment and react to different stimuli and changes in the environment. These reactions can either be based on transcriptional activity or chemical modifications of existing proteins, so called post-translational modifications. In order to gain insight into the regulation of these protein-interaction networks it is necessary to identify proteins, their modifications and to measure the protein expression levels within a cell. This requires the identification of several hundreds or thousands proteins and their quantification in a complex mixture. In addition the information has to be collected in a time efficient way. All these requirements are matched by modern mass spectrometry and result in the methods rise to be the default method for large-scale protein identification in life sciences.

The core facility mass spectrometry offers a wide range of mass spectrometry methods for the identification and quantification of proteins and peptides. Besides the identification of proteins in gel slices, the mass spectrometry core facility uses a number of proteomic techniques in different collaborations with groups at the MDC (Figure 1). We are working closely with these groups to optimize the methods for the different projects.

Fig 1: *Electro spray setup (ESI) for the analysis of peptides. Peptides are ionized in front of the orifice of the mass spectrometer. The charged peptides are measured in the mass spectrometer.*



Targeted proteomics

Monitoring the regulation of proteins under different conditions can provide deeper insight in the regulatory network, which underlies a signal transduction cascade. For many cascades the major players in these pathways are already identified. Incorporating this knowledge into a targeted strategy allows focusing to monitor changes in the concentration of these players, avoiding the sequencing of unrelated, not regulated proteins in the cell. A method that allows the selection of a limited number of proteins is selected reaction monitoring (SRM). The technique has the advantage of a high sensitivity combined with short run times on the liquid chromatography systems. This opens the possibility of measuring large quantities of different samples in a short time period and quantifying all components of the cascade.

Several collaborations of the core facility within the MDC are now based on multiple reaction monitoring experiments. The core facility has access to two different mass-spectrometers capable for this type of experiments (Q-Trap 5500 and Q-Trap 4000).

Non-targeted proteomic approaches

Metabolic labeling of cells offers great advantages for the quantification of proteins. The cells of interest are therefore cultured in media, which contain isotopic labeled (non radioactive) amino acids (Figure 2). The additional mass of the amino acids can later be detected by mass spectrometry. Comparison of two different samples is possible by analyzing the sample on a high accuracy mass spectrometer (LTQ-Orbitrap). The results of these measurements are relative ratios for each protein detected in the two different samples. This techniques is currently used by the core

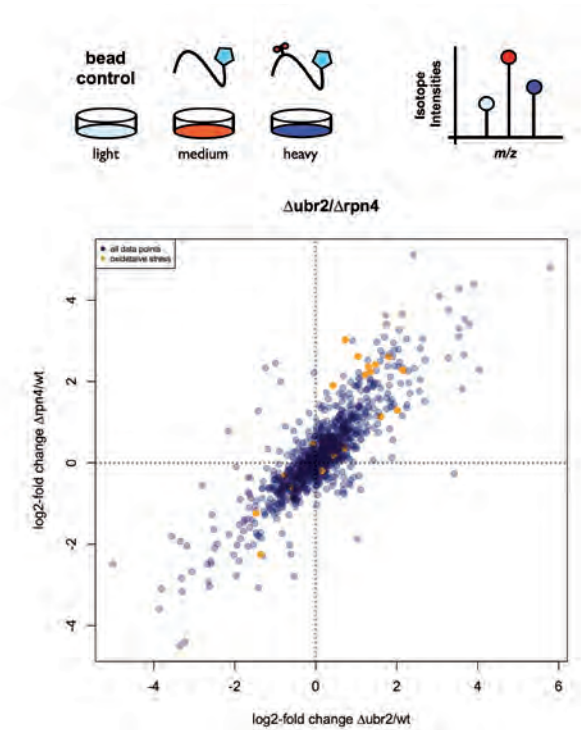


Fig 2: *Metabolic labelling of proteins using isotope containing amino acids allows the quantification of several thousand proteins in one experiment. The isotope containing amino acids are fed to cells in cell culture (SILAC) leading to a homogeniously labelled population of proteins. After mixing the differently labelled proteins and preparing them for the analysis the peptide mixtures are analyzed using a high resolution mass spectrometer. The spectra contain characteristic pairs of mass peaks which can be used by the analysis software for the quantificaiton of the samples. The SILAC technique can be applied to the analysis of complex protein mixtures (lower panel) or the analysis of the proteins interactome (upper panel).*



Fig 3: *Analysis of digestion patterns generated by the proteasome a 2 MDa protease. The digestion of foreign proteins is important for the cellular immunity. The proteasome as one of the largest cellular proteases generates peptides from proteins, which can then presented by the immun system. The analysis of the peptides generated by the proteasome in vitro were analysed and revealed new interesting digestion patterns*

facility for the quantification of immuno-precipitations and large scale protein identifications.

For samples, which cannot be cultured in media containing heavy amino acids, other techniques are available. This techniques either rely on a highly reproducible liquid chromatography (Figure 3) or the peptides are labelled post-lysis using a chemical reaction.

Identification of post-translational modifications

Besides the translational regulation of proteins, another layer of regulation exists, which is mediated by post-translational modifications. For the understanding of the molecular mechanisms, which regulate these proteins, it is necessary to gain insight into the different post-translational modifications of proteins. The core facility actively pursues the development of new methods for the identification of modification sites by ubiquitin-like proteins.

Selected Publications

Bagola, K., von Delbrück, M., **Dittmar, G.**, Scheffner, M., Ziv, I., Glickman, M., Ciechanover, A. and Sommer, T. Ubiquitin binding by a CUE domain regulates ubiquitin chain formation by ERAD E3 ligases, **Molecular Cell**, 50, 528-539 (2013).

Anton, F., **Dittmar, G.**, Langer, T. & Escobar-Henriques, M. Two deubiquitylases act on mitofusin and regulate mitochondrial fusion along independent pathways. **Molecular Cell**, 49, 487-498 (2013).

Müller-Rischart, A.-K., Pilsl, A., Beaudette, P., Patra, M., Hadian, K., Funke, M., Peis, R., Deinlein, A., Schweimer, C., Kuhn, P.-H., Lichtenthaler, S., Motori, E., Hrelia, S., Wurst, W., Trümbach, D., Langer, T., Krappmann, D., **Dittmar, G.**, Tatzelt, J., and Winklhofer, K.. The E3 ligase parkin maintains mitochondrial integrity by increasing linear ubiquitination of NEMO. **Molecular Cell**, 49, 908-921 (2013).

Stock, K., Kumar, J., Synowitz, M., Petrosino, S., Imperatore, R., St J Smith, E., Wend, P., Purfürst, B., Nuber, U.A., Gurok, U., Matyash, V., Wälzlein, J.-H., Chirasani,, S.R., **Dittmar, G.**, Cravatt, B.F., Momma, S., Lewin, G.R., Ligresti, A., De Petrocellis, L., Cristino, L., Di Marzo, V., Kettenmann, H., and Glass, R. Neural precursor cells induce cell-death of high-grade astrocytomas via stimulation of TRPV1. **Nature Medicine**, 18, 1232–1238 (2012).

Schwanhäusser, B., Busse, D., Li, N., **Dittmar, G.**, Schuchhardt, J., Wolf, J., Chen, W., and Selbach, M. Global quantification of mammalian gene expression control. **Nature** 473, 337–342 (2011).

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Photo: Peter Himsel/MDC

Arnd Heuser

Pathophysiology

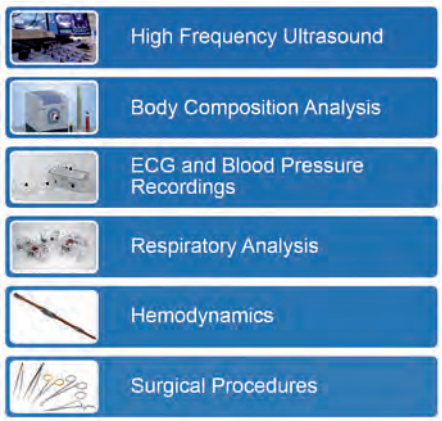
Despite numerous advances in research techniques such as in vitro methods and advanced computer modeling that avoid the use of animals, there is still the need for a whole-system approach to preclinical research and to obtaining data from conscious and unconscious animals. For example, understanding the complex physiological interactions between the cardiovascular, respiratory and central nervous systems will be critical for the development of many human pharmaceuticals and therapies.

The Pathophysiology Platform provides researchers with a central resource for the study of animal disease models. We use validated approaches and state-of-the-art instrumentation that allow for the sensitive screening of phenotypic variations. We focus on techniques which minimize animal use and enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals, thereby reducing animal use in the future. With this aim, we use a variety of non-invasive in vivo test tools that allow the monitoring of physiological and bioelectrical variables (e.g. blood pressure, heart rate, ECG and respiration) in conscious animals. The non-invasive, whole-body imaging of small animals using techniques such as High-Frequency Ultrasound and Time-Domain-Nuclear-Magnetic-Resonance imaging is helping to reduce the number of animals used in basic research and drug development. The same animal can be imaged multiple times in order to monitor visually, often in real time, the progression or regression of infections or disease. This avoids the need to sequentially sacrifice animals at different time points, allowing for significant reductions in the number of animals used per study. We continuously try to improve scientific procedures and husbandry to minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. While the central focus of the facility is cardiovascular research, the techniques that are employed may also be useful to investigators in other fields such as cancer, neurobiology and developmental biology.

High Frequency Ultrasound

Ultrasonography is a powerful imaging method that permits a noninvasive, real-time visualization of organs and tissues. We perfom sonographic studies of small animals using a High-Frequency Ultrasound system. Its transducers produce frequencies as high as 70MHz, permitting the identification of structures as small as 30µm with a maximum temporal resolution of 740fps. This makes the platform ideal for pre-clinical research because it permits

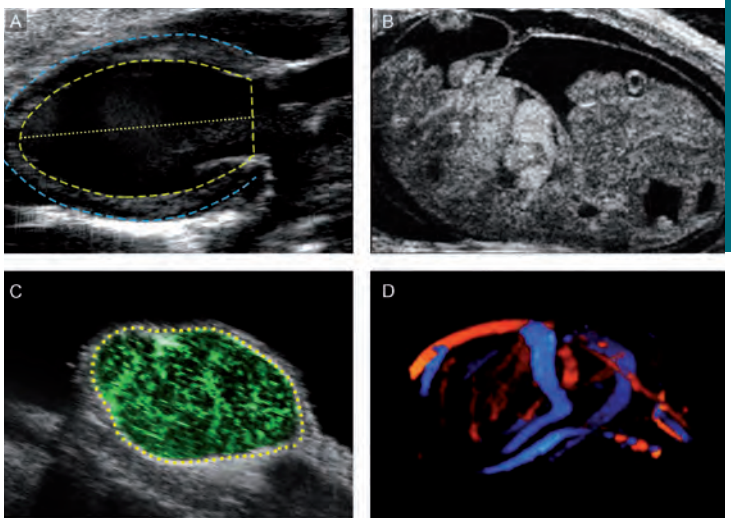
The Methodological Spectrum of the Pathophysiology Platform.



the study of many organs, tissues, and the behavior of biological systems that need to be studied at this tiny scale. The non-invasive nature of ultrasound means that it can be used without harming animal or human subjects. We have established a wide variety of protocols to analyze the phenotypes of mice and rats: For cardiovascular studies, ultrasound imaging can be used to detect the presence of localized or generalized hypertrophy or a thinning of the myocardium of the left ventricle (LV) and the presence of regional or global wall motion abnormalities associated with systolic dysfunction. The application of transmitral Doppler analysis permits the detection of abnormal filling patterns associated with LV diastolic dysfunction. Strain analysis provides a highly sensitive speckle-tracking-based echocardiographic imaging technique that offers a quantitation of the velocity of the walls, displacement, strain, strain rate, and time to peak analysis. Because of its non-invasive nature, ultrasound can be used in longitudinal studies for tumor diagnosis and monitoring during therapies. 3D-Ultrasound imaging allows for a reliable delineation of tumor boundaries and an assessment of tumor heterogeneity. In addition, ultrasound imaging is ideal for studying pregnancy and development in mice and rats.

Body Composition Analysis

Time-Domain-Nuclear-Magnetic-Resonance imaging (TD-NMR) is a powerful tool for the non-invasive quantification of solids and liquids in objects under study. Without anesthesia or other preparations, animals such as mice and rats can be tested for body composition of fat, free body fluids and lean tissue within a few minutes. After a stimulated adjustment of nuclear particle spins within a high energetic magnetic field, specific patterns of relaxation times of spin rearrangements are recorded upon removal of the magnetic field. These relaxation patterns can be correlated to the density of body compounds, i.e. lean tissue, fat and free fluids, giving concrete values of their proportional composition. The NMR-based body composition analyzer allows non-invasive and reliable quantifications of whole-body components of small animals.



High Frequency Ultrasound Imaging:
(A) Parasternal Long Axis View of a Murine Heart,
(B) Micro-Ultrasound Imaging of a Murine Embryo at E12.5,
(C) Contrast Imaging of a Melanoma Tumor,
(D) 3D-Color Doppler of a Murine Testicle.

Selected Publications

alpha(1A)-adrenergic receptor-directed autoimmunity induces left ventricular damage and diastolic dysfunction in rats. Wenzel, K. and Wallukat, G. and Qadri, F. and Huebner, N. and Schulz, H. and Hummel, O. and Herse, F. and Heuser, A. and Fischer, R. and Heidecke, H. and Luft, F.C. and Mueller, D.N. and Dietz, R. and Dechend,R. PLoS ONE 5 (2): e9409. 24 February 2010

Growth arrest specific protein 6 participates in DOCA-induced target-organ damage. Park, J.K. and Theuer, S. and Kirsch, T. and Lindschau, C. and Klinge, U. and Heuser, A. and Plehm, R. and Todiras, M. and Carmeliet, P. and Haller, H. and Luft, F.C. and Mueller, D.N. and Fiebeler, A. Hypertension 54 (2): 359-364. August 2009

Role of the multidomain protein spinophilin in blood pressure and cardiac function regulation.da Costa-Goncalves, A.C. and Tank, J. and Plehm, R. and Diedrich, A. and Todiras, M. and Gollasch, M. and Heuser, A. and Wellner, M. and Bader, M. and Jordan, J. and Luft, F.C. and Gross, V. Hypertension 52 (4): 702-707. October 2008

Dietary n-3 polyunsaturated fatty acids and direct renin inhibition improve electrical remodeling in a model of high human renin hypertension. Fischer, R. and Dechend, R. and Qadri, F. and Markovic, M. and Feldt, S. and Herse, F. and Park, J.K. andGapelyuk, A. and Schwarz, I. and Zacharzowsky, U.B. and Plehm, R. and Safak, E. and Heuser, A. and Schirdewan, A. and Luft, F.C. and Schunck, W.H. and Mueller, D.N. Hypertension 51 (2): 540-546. February 2008

Soluble epoxide hydrolase is a susceptibility factor for heart failure in a rat model of human disease. Monti, J. and Fischer, J. and Paskas, S. and Heinig, M. and Schulz, H. and Goesele, C. and Heuser, A. andFischer, R. and Schmidt, C. and Schirdewan, A. and Gross, V. and Hummel, O. and Maatz, H. and Patone, G. andSaar, K. and Vingron, M. and Weldon, S.M. and Lindpaintner, K. and Hammock, B.D. and Rohde, K. and Dietz, R.and Cook, S.A. and Schunck, W.H. and Luft, F.C. and Huebner, N. Nature Genetics 40 (5): 529-537. May 2008

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Photo: David Ausserhofer/MDC

Hans-Peter Rahn

Preparative Flow Cytometry

Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry. It was designed in 1971 by Len Herzenberg to provide a fast physical separation method to fractionate heterogeneous mixtures of cells into distinct subsets, based upon the light scattering and fluorescent characteristics of different cell types. The FACS Core Facility at the MDC mainly provides assistance in operating cell sorters with a speed up to 20,000 cells per second for large-scale cell isolation; for more analytical purposes, single cells can also be spotted very precisely into microtiterplates or onto slides. In addition, the core facility staff assists in the design and set-up of sophisticated experiments on additional state of the art analyzers. The facility operates one analyzer of the type "BD LSR", one 4-colour laser line sorter BD Fortessa, and three high-capacity "BD Aria" digital sorters.

The facility has been used over the last few years by many different scientific groups from the MDC, BIMSB, ECRC and the FMP. Sorted cells are often prepared for microarray- or sequencing-based gene expression analysis, quantitative real-time PCR, DNA sequencing, live-cell imaging or adoptive transfer experiments in animals.

The greatest number of requests for all preparative cell sorting at the MDC arise from primary human and murine cells, which are characterized by their immunophenotypic parameters. For example, the group of Bernd Dörken routinely uses FACS enrichment of tumor and primary murine hematopoetic cells in order to analyse the function of ectopically expressed genes in comparison to endogenous expression levels in lymphoma cells.

The research group of Klaus Rajewsky has generated various mouse models of human lymphomas and fatal lymphoproliferative disorders to elucidate underlying mechanisms of pathogenesis as well as genetically manipulated mice for conditional loss- or gain-of-function studies to discover the role of specific gene elements. They have been developing a new genome editing technology, the CRISPR/Cas9 system. The FACS analyzer and sorter are indispensable equipment to characterize, quantify, and purely isolate specific immune cell population as well as genetically modified cells. The BD Fortessa equipped with 14-channel parameters enables them to analyze extremely rare cell population such as haematopoietic stem cells, memory cells, and cancer progenitors.

Stromal cells are pivotal for the onset and survival of lymphoma B cells; however, which stromal cell subpopulations within lymph nodes contribute to the formation of a lymphoma growth-promoting niche remained elusive. The group of Armin Rehm has applied a combination of magnetic bead cell enrichment and FACS sorting to isolate rare stromal subsets of mesenchymal origin which could be used for gene expression analysis. Thus, the establishment of this method was a prerequisite to the analysis of a specific tumor-induced gene expression profile in endothelial and other stromal cell types.

In addition, the group of Martin Lipp focuses on distinct memory and effector T cell populations which are important for the

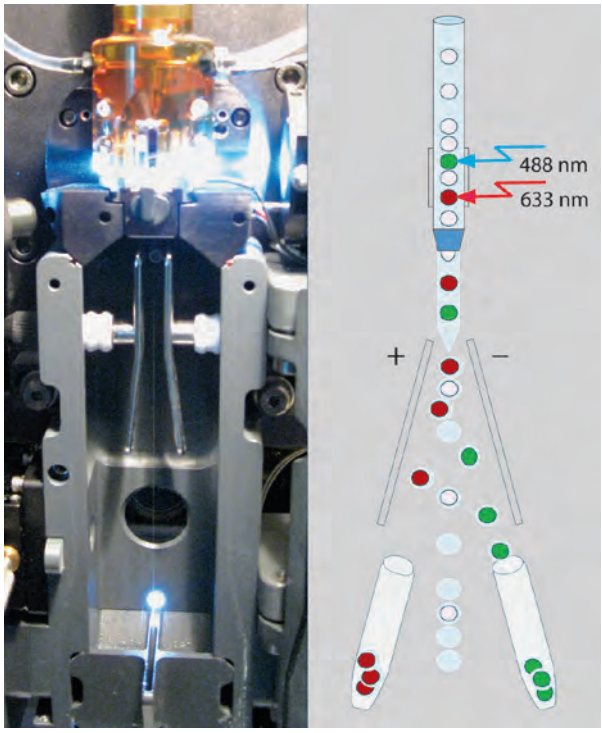
development of antibody-based adaptive immune responses. In this context central memory, effector/memory and follicular B helper T cells are enriched by FACS using specific patterns of surface markers on these cells.

More recently, FACS technologies have gained substantial importance for the group of Walter Birchmeier; in this lab, the focus has been on the analysis and isolation of tumor and benign stem cells. For example, it is possible to separate somatic stem cells of the skin, the mammary gland, the heart, and neuronal stem cells. Reliable cell-surface biomarkers are becoming more widely available. This holds true also for the area of cancer stem cells or cancer-initiating cells.

Sorting is also used by the lab from Carmen Birchmeier to study how different signaling pathways control the development and function of satellite cells, the stem cells of adult muscle. These cells are needed for muscle growth and also for the regeneration of injured muscle tissue. Although satellite cells account for only a small fraction of muscle tissue, they can be isolated and enriched by FACS, permitting an analysis of gene expression changes in mouse mutants and a more detailed characterization of these cells using in vitro assays.

FACS technology has also gained impact at the MDC in the generation of iPS (induced pluripotent stem) cells. For example, the group of Mathias Treier labels ES cells with various reporters containing fluorescent proteins to enrich different germ layers during differentiation to understand how cell fate decisions are made.

To study early embryogenesis employing high-throughput genomics as well as biochemistry assays, the lab of Nikolaus Rajewsky devised a method to collect large amounts of precisely staged *C. elegans* embryos by FACS. This method will make a contribution towards a more complete understanding of gene regulatory networks during early *C. elegans* development.



Left: Inside view of a FACSAria (flow cell with sort block and deflection plates), Right: Schematic view of the separation principle

Selected Publications

Mathas, S., S. Kreher, K.J. Meaburn, K. Jöhrens, B. Lamprecht, C. Assaf, W. Sterry, M.E. Kadin, M. Daibata, S. Joos, M. Hummel, H. Stein, M. Janz, I. Anagnostopoulos, E. Schrock, T. Misteli, and B. Dörken. 2009. Gene deregulation and spatial genome reorganization near breakpoints prior to formation of translocations in anaplastic large cell lymphoma. Proceedings of the National Academy of Sciences of the United States of America. 106:5831–6. doi:10.1073/pnas.0900912106.

Bröske, A.-M., L. Vockentanz, S. Kharazi, M.R. Huska, E. Mancini, M. Scheller, C. Kuhl, A. Enns, M. Prinz, R. Jaenisch, C. Nerlov, A. Leutz, M. a Andrade-Navarro, S.E.W. Jacobsen, and F. Rosenbauer. 2009. DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. Nature genetics. 41:1207–15. doi:10.1038/ng.463.

Klaus, A., M. Muller, H. Schulz, Y. Saga, J.F. Martin, and W. Birchmeier. 2012. Wnt/-catenin and Bmp signals control distinct sets of transcription factors in cardiac progenitor cells. Proceedings of the National Academy of Sciences. 109:10921–10926. doi:10.1073/pnas.1121236109.

Bröhl, D., E. Vasyutina, M.T. Czajkowski, J. Griger, C. Rassek, H.-P. Rahn, B. Purfürst, H. Wende, and C. Birchmeier. 2012. Colonization of the Satellite Cell Niche by Skeletal Muscle Progenitor Cells Depends on Notch Signals. Developmental Cell. 23:469–481. doi:10.1016/j.devcel.2012.07.014.

Stoeckius, M., J. Maaskola, T. Colombo, H.-P. Rahn, M.R. Friedländer, N. Li, W. Chen, F. Piano, and N. Rajewsky. 2009. Large-scale sorting of C. elegans embryos reveals the dynamics of small RNA expression. Nature methods. 6:745–751.

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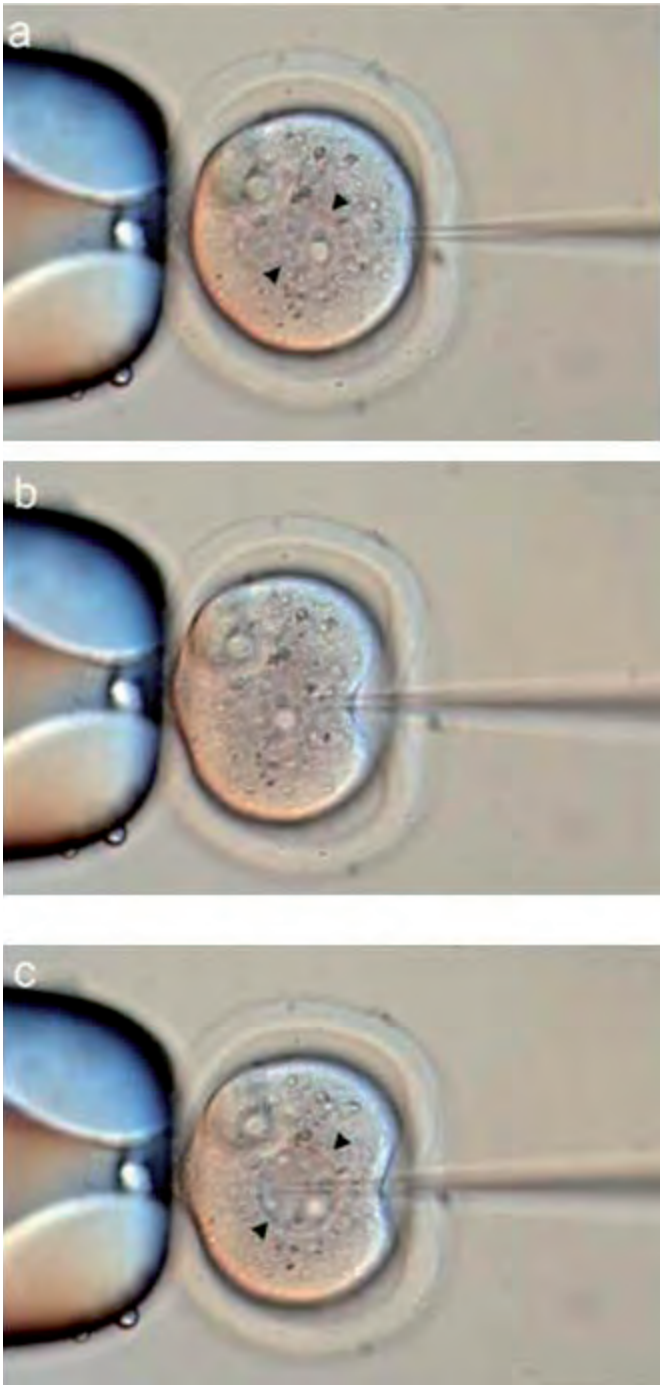
(From May 2014):
Ralf Kühn

Transgenics

Transgenic Core Facility (TCF)

The Transgenic Core Facility (TCF) offers expertise in all kinds of mouse Assisted Reproductive Technologies (ART). Starting at the planning phase, we support scientists from the MDC and their external collaborators with the design and layout of their projects. One focus of our work is the generation of genetically modified mouse lines. This is accomplished either by gene targeting in embryonic stem (ES) cells and subsequent generation of chimeric mice from recombinant ES cell clones or by micro-injection of plasmid or BAC type transgenes into the pronuclei of fertilized oocytes.

A second focus of our work is the rederivation of lines for the population of the new very clean facility with highest hygienic demands that allows the breeding of immune deficient mouse lines without the burden caused by opportunistic infections. Moreover we cryo-preserve precious mouse lines and bring frozen lines back to life. Since most groups choose to conserve mouse sperm this is the main form of germ plasm we freeze down and store in liquid nitrogen. Due to the growing number of mouse models worldwide, we see an increase in the number of organizations who offer to send frozen material instead of live mice.



Pronuclear microinjection. When a zygote is fixed to the holding capillary, both the injection needle filled with DNA solution as well as one of the pronuclei are brought into focus. Then the zona pellucida is penetrated with the injection capillary aiming at one of the pronuclei (a). The injection capillary is moved further to the far side of the pronucleus and then slightly pulled back to place the capillary's tip in its middle (b). When the injection pressure is applied a swelling of the injected pronucleus has to be clearly visible (comparearrowheads in a to those in c).

These lines are revitalized at the MDC following a variety of protocols depending on the method of cryo-preservation. There are individual projects that do not fit into any of the above that can be supported by the TCF and we will help whenever it comes to the production, isolation, manipulation, culture or retransfer of pre-implantation embryos. Moreover, we can give advice on cloning and targeting strategies, BAC preparation, ES cell culture, ES cell strain background, coat color genetics, ES cell derivation, and help with the selection of targeted ES cell clones from international knock out consortia for the generation of the corresponding gene targeted mouse lines. A future focus of the TCF will lie on the utilization of new technologies for genome modification via enzyme mediated induction of double strand breaks and subsequent homologous recombination either in cells or in zygotes directly.

Selected Publications

Transposon-mediated transgenesis, transgenic rescue, and tissue-specific gene expression in rodents and rabbits. Katter K, Geurts AM, Hoffmann O, Mátés L, Landa V, Hiripi L, Moreno C, Lazar J, Bashir S, Zidek V, Popova E, **Jerchow** B, Becker K, Devaraj A, Walter I, Grzybowksi M, Corbett M, Filho AR, Hodges MR, Bader M, Ivics Z, Jacob HJ, Pravenec M, Bosze Z, Rülicke T, Izsvák Z. FASEB J. 2013 Mar;27(3):930-41. doi: 10.1096/fj.12-205526. Epub 2012 Nov 29.

Record 2013:

- **13 lines from recombinant ES cells**
- **52 sperm freezings**
- **6 in vitro fertilizations**
- **13 hygienic rederivation**

Group Leader
PD Dr. rer. nat. Ralf Kühn
(From May 2014)

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Technical Assistants
Katja Becker
Andrea Leschke



Technology Transfer

Technology Transfer

Technology transfer at the Max Delbrück Center aims to identify, protect, manage and market promising findings and technologies resulting from research. This entails, for example, supporting the MDC's scientists in drafting patenting and marketing strategies and consulting them on whether an invention would be best advanced by licensing, by establishing a cooperation agreement with industry, or by founding a start-up company. The MDC has established technology transfer activities and relations with industry partners.

At the MDC, technology transfer is coordinated by the legal and technology transfer department and carried out in collaboration with an external company, Ascenion GmbH, which provides scientific advice for inventions and the patenting and commercialization process. Ascenion is the daughter company of the German Life Science Foundation, which the MDC founded together with the other Helmholtz research centers, i.e. the German Research Center for Environmental Health, the Helmholtz Centre for Infection Research and the German Cancer Research Center.

As is the case with most basic research, the MDC's inventions and discoveries often need substantial further development before they can enter the market. Despite significant progress in the elucidation of disease pathways and novel drug targets, a major bottleneck still lies in identifying and producing substances that can potentially be used for therapies. To this end, the MDC builds collaborations between the MDC, the Leibniz-Institut für Molekulare Pharmakologie (FMP) on the Berlin-Buch campus, the European Molecular Biology Laboratory (EMBL) and further Helmholtz institutes. These activities draw together expertise from the partners to establish new platforms for drug screening, to improve hits through medicinal chemistry methods, and provide new animal models for in vivo validation.

Several funding schemes exist to help bridge the gap between basic research and commercialization of inventions, e.g. the BMBF Validation Fund, the BMBF Go-Bio-Program or the Helmholtz Validation fund. In addition, the MDC has set up an internal funding program. Projects funded under this scheme demonstrate innovative commercial or clinical potential and are likely to obtain external funding once further developed. Also, MDC funding can be used to follow up expired Go-Bio awards, thus supporting later implementation phases. Hence, the MDC's program strengthens the institute's own applied and spin-off oriented research and facilitates moving from research into industry.

The MDC technology transfer strategy refers to international benchmarks such as invention disclosures, patent applications, patent assignments, cooperation with industry, license agreements and license proceeds as a measure of its success. On average, the MDC files 10-15 invention disclosures per year, the majority of which lead to a patent. Currently, the MDC holds around 70 patent families with over 300 patents. The MDC's most successful area of technology transfer is the commercialization of animal models and of biological materials. At present, 8 active projects are funded by the

internal MDC technology transfer program. Key to successful licensing of technologies are an effective patent strategy, excellent publications and well-established contacts to the industry. It is very important that the scientists are sensitized and supported to file patents before they submit their paper to journals. It has to be ensured, however, that the patenting procedure does not delay the publication. At every point in the patenting process the question of the potential licensee needs to be addressed. During the whole patenting process, especially at every decisive point (such as wording of the patent application for rule example; the decision to nationalize the application), it must be considered who can be a potential licensee.

The MDC also supports scientist-initiated spin-offs. In this area the cooperation with Ascenion is close. Ascenion develops a special program "Spinnovator", where private and public money together finance spin-offs. The successful MDC spin-off OMEICOS is the beneficiaries of the program.

Figures for Technology Transfer 2013

Patent applications per year	4
Patent rights	305
License agreements (new in 2013)	2
License revenues (in TEUR)	195
Company foundings	1

Annett Böddrich

Enabling Technologies for Drug Discovery in Alzheimer's and Further Protein Misfolding Diseases

Annett Böddrich, Thomas Wiglenda, Sandra Neuendorf, Lydia Brusendorf, Nancy Schugardt, Sigrid Schnögl, Erich E. Wanker

Alzheimer's, Parkinson's and Huntington's disease are neurodegenerative disorders, for which no effective treatments exist. The diseases are believed to be caused by misfolding and aggregation of disease-relevant polypeptides. We have started to establish a technology platform for the identification and characterisation of chemical and biological substances that interact with the protein misfolding and aggregation cascade of different disease proteins. In the last year, we successfully established a small molecule screening assay for the amyloid-beta target in Alzheimer's disease. The assay is based on the time-resolved Förster's resonance energy transfer (TR-FRET) technology. This technology relies on a quantifiable FRET signal that can be measured when amyloid-beta forms aggregates in vitro. Applying it, compound effects can be detected with high sensitivity and specificity. To validate the assay, parameters like the Z-factor were determined, indicating suitability for high-throughput screening of large compound libraries. The assay was successfully automated and applied to a focussed library of 1,200 small molecule compounds, which consisted of about 500 compounds we previously discovered to act on aggregation of various misfolding-prone proteins and of the library of FDA-approved compounds. Currently, we are identifying new funding sources and industry-based collaboration partners to broaden the technology platform and continue characterisation of hit compounds.

Adeeb Eldahshan

Development of inhibitors of intracellular protein-protein interactions for the treatment of heart failure

Adeeb Eldahshan, Sylvia Niquet

Protein-protein interactions represent a new class of drug targets and their inhibition may lead to the discovery of novel drugs that are more effective and have fewer side effects than conventional drugs. In this context, we focus on A-kinase anchoring protein (AKAP)-dependent protein-protein interactions that play a crucial role in heart failure. AKAPs comprise a family of about 50 scaffolding proteins that tether signalling proteins to defined cellular compartments. All AKAPs bind protein kinase A (PKA) and additionally bind various other kinases, their substrates, phosphatases and phosphodiesterases. Thus, they form signalling hubs and coordinate cellular signalling spatially and temporally. Our aim is to develop previously identified small molecule inhibitors of AKAP-dependent protein-protein interactions towards drug candidates for the treatment of heart failure.

In our medicinal chemistry laboratory we optimise these molecules with regard to specificity and affinity. Therefore, we develop diversity-oriented synthesis concepts and test the structure-activity-relationships (SAR) of the molecules *in vitro.* We use a variety of techniques, including molecular modelling, Nuclear Magnetic Resonance (NMR), specifically ^{1}H-^{15}N heteronuclear single quantum correlation (HSQC), computational screening as well as biacore and homogenous time-resolved fluorescence (HTRF) assays. These efforts are strongly connected to the research of the Anchored Signalling group of Prof. W. Rosenthal and PD Dr. E. Klußmann that tests novel molecules with regard to biological activity.

Christiane Wetzel and Gary R. Lewin

Exploitation of novel transduction targets for pain relief

Christiane Wetzel, Gary R. Lewin, Kathleen Barda

There is an unmet need for more effective analgesics and novel strategies for analgesic drug development. With Stomatin-like protein-3 (STOML3) we have identified a potential molecular target that directly participates in the transduction of noxious and innocuous mechanical stimuli in sensory neurons. We found that STOML3 deficient mice have many mechanoreceptors and nociceptors which are essentially insensitive to mechanical stimulation. Interestingly, STOML3 deficient mice show only very minor symptoms of neuropathic pain and this may be due to the impairment of touch reception in these mice. We are developing novel high throughput screens for small molecules that disrupt STOML3 function.

For further validation and lead optimization of small molecules we employ a set of experimental paradigms. For example we can test whether candidate compounds block mechanosensitive currents in isolated sensory neurons using the whole-cell patch-clamp technique. We can further test if local application of our compounds to the skin will block mechanosensitivity at the receptive endings of single primary afferents in the skin using the *in vitro* skin-nerve technique. Eventually we will perform behavioral tests in order to determine if the application of novel, potentially analgesic compounds lead to a lack of hypersensitivity to touch evoked pain in an animal model of neuropathic pain.

David Miles Carter

Development of novel compounds that inhibit growth of prostate cancer cells

David Carter, Udo Heinemann, Ulrich Gohlke *(MDC),* **Edgar Specker, Jessica Przygodda, Marc Nazaré** *(FMP)*

Histone lysine demethylase (KDM) enzymes are an emerging class of therapeutic targets, known for their ability to regulate the growth of various cancer cells. The KDM4 family of isoenzymes comprises six members denoted KDM4A-F. Both KDM4A and KDM4C isoforms are known to regulate the growth of prostate cancer (PCa) cells via an epigenetic mechanism. The enzymes stimulate gene transcription by demethylating tri- and di-methylated forms of lysine 9, within the core histone H3. This process initiates the expression of various growth factors, which facilitate the growth of cancer cells. Knockdown of both isoforms was shown to halt growth of PCa cell lines and demonstrated their novelty as targets for development of small molecule inhibitors, which may prove useful in a clinical setting. To address this possibility, we performed a high-throughput screening campaign in order to identify compounds that inhibit the most active KDM4 isoform, KDM4E. We identified several compounds that inhibit KDM4E with IC50 values in the mid- to low-micromolar range. Importantly, the compounds specifically inhibit the KDM4 family of enzymes and show reduced activity towards other representative members of the KDM superfamily. Currently we are employing methods of structure-based drug design to increase the potency and selectivity of these compounds. In parallel, we are establishing cell-based assays to monitor the ability of our compounds to halt or slow the growth of PCa cell lines. Ultimately we aim to test the efficacy of our compounds in a mouse xenograft model of PCa and to steer the compounds towards completion of pre-clinical studies.

Michael Radke

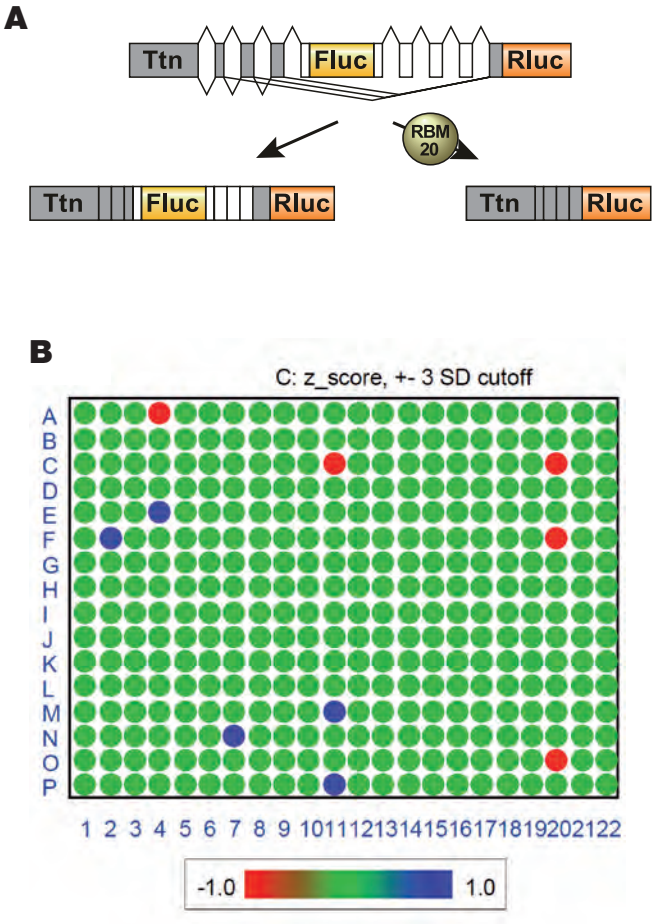
Alternative Splicing as a Therapeutic Target for Cardiac Disease

Michael Radke *(Scientist)*, **Carmen Judis** *(Technician)*, **Michael Gotthardt, Martin Liss, Florian Hinze, Jens Peter von Kries (FMP Berlin – Screening Unit)** *(Associated Scientists)*

Cardiovascular diseases are the main cause of death in developed countries. While several pharmacological agents have been developed that help improve cardiac contraction (systolic function), there is no targeted therapy to improve cardiac filling (diastolic function). Heart disease with preserved ejection fraction (alias diastolic dysfunction) is more prevalent in females. Risk factors are hypertension, diabetes and increased age of the patient, but the molecular basis of diastolic heart disease is only poorly understood.

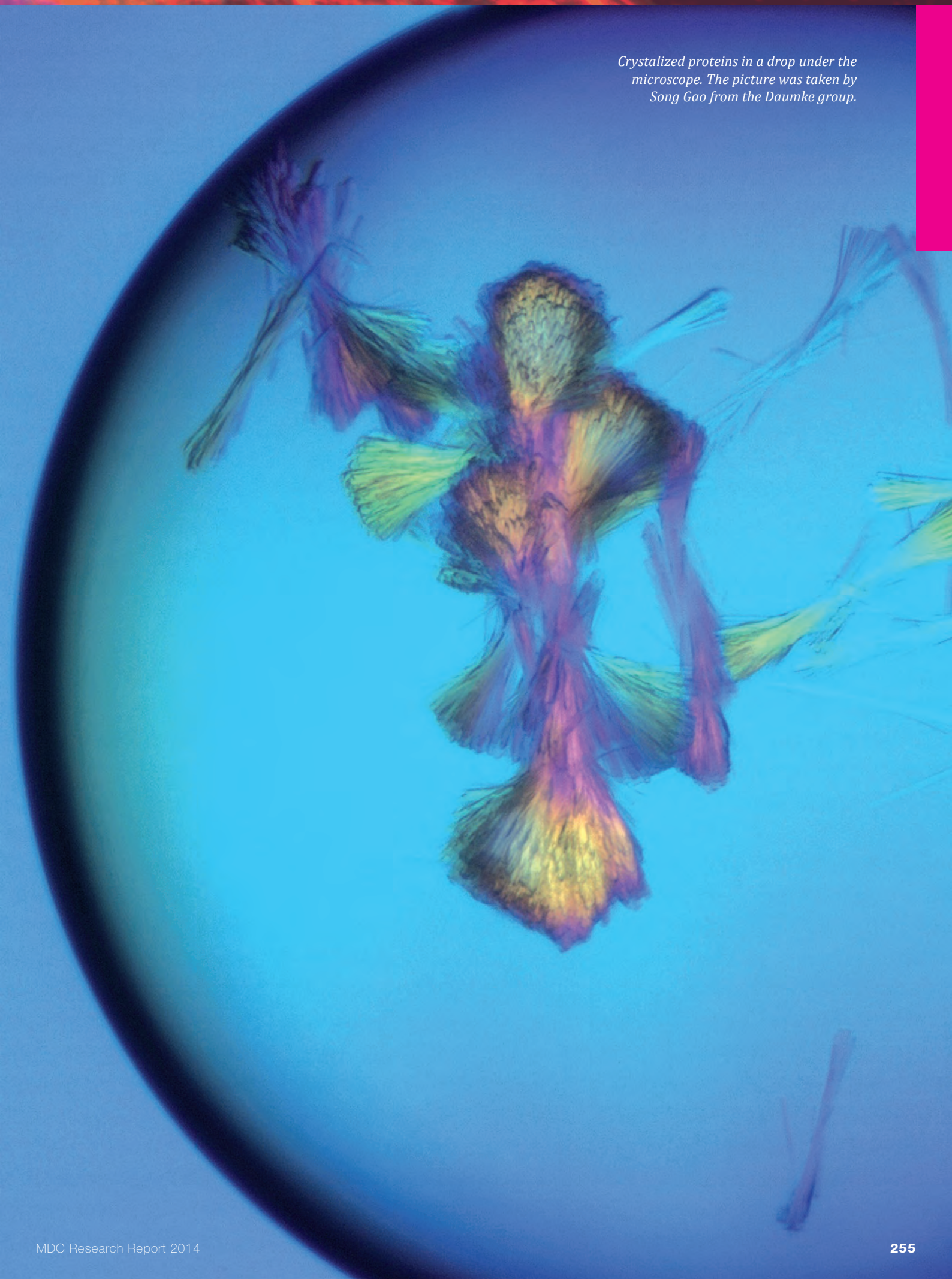
The elastic properties of the heart and thus diastolic filling are largely determined by the extracellular matrix, the sarcomere, and proteins regulating ion traffic. Most of these proteins are alternatively spliced to concertedly adapt cardiac filling. Deficiency of select factors that regulate splicing can result in genetic forms of cardiac disease. Furthermore, both cardiomyopathies and coronary artery disease of unknown cause have been associated with differential isofom expression of sarcomeric proteins such as titin. Therefore alternative splicing could be a suitable therapeutic target.

We use *in silico* analysis, cell based reporter assays, and animal models to identify splice factors that regulate cardiac isoform expression. Among them is RBM20, which regulates alternative splicing of titin as well as other sarcomeric and signaling proteins related to diastolic function[1]. Using an RBM20 dependent reporter assay and high throughput screening we aim to identify small molecules that modulate alternative splicing. These are evaluated for toxicity, and validated in tissue culture and in animal models, such as a genetic model with diastolic dysfunction, as a prerequisite for future clinical studies.



Reporter assay to screen for splice activators and inhibitors.
A) Titin based splice reporter assay. Without RBM20, both firefly (Fluc) and renilla luciferase (Rluc) are included in the resulting isoform. With RBM20, the exon containing Fluc is excluded. B) Small molecule screen to identify splice effectors. Potential splice inhibitors (red), splice activators (blue), or compounds that do not influence splice activity (green) are derived from a screen of 40.000 compounds in the 384-well format

1. Guo, W. et al. RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. Nat. Med. 18, 766–773 (2012).



Crystalized proteins in a drop under the microscope. The picture was taken by Song Gao from the Daumke group.

Academics

Akademische Aktivitäten



International PhD Program

Internationales Doktorandenprogramm

The MDC considers the training of new generations of researchers in Molecular Medicine to be a prerequisite for sustainable development and international scientific success. The PhD program combines biomedical research and interdisciplinary training, complemented by a career development program, and provides high-end technology and project opportunities.

In 2003, the MDC and the Humboldt-Universität zu Berlin (HU) established the *International PhD Program* to attract talented international graduate students. Receiving over 1000 applications annually, the *International PhD Program* invites around 100 candidates from all over the world for on-site assessment in Berlin. Every year 50-100 PhD students begin their thesis in one of the research groups at the MDC or its partners HU, Freie Universität (FU), Charité –Universitätsmedizin Berlin, or the Leibniz Institut für Molekulare Pharmakologie (FMP). More than 40% of these doctoral students come from abroad, representing 36 countries.

Following the success of the *International PhD Program,* the MDC and its university partners were awarded a grant from the Helmholtz Association to establish the *Helmholtz Graduate School "Molecular Cell Biology" (HGS-MCB)* in 2007. The *HGS-MCB* offers an interdisciplinary platform for structured scientific training and outstanding opportunities for personal development of doctoral students. Currently, 350 students work towards their PhD degree at the MDC and benefit from the academic curriculum. Training records of individual students reflecting their personal needs and interests are documented in the *HGS-MCB* credit point system, which is the basis of the PhD certificates students receive upon completion of their degree.

The *HGS-MCB* offers:
- A Welcome Center to help international students settle in Berlin
- A University interface
- Supervision by a PhD Thesis Committee and regular meetings to evaluate students' progress
- Scientific training: lectures and seminar series in general molecular medicine and specialized areas, courses and introduction into new methodologies, good scientific practice
- Professional skills training and career development complementing the scientific training

Die Ausbildung des wissenschaftlichen Nachwuchses in der Biomedizin ist eine grundlegende Voraussetzung für die nachhaltige Entwicklung und den internationalen wissenschaftlichen Erfolg des MDC. Das *Internationale Doktorandenprogramm* verbindet die Weiterbildung junger Nachwuchstalente mit biomedizinischer Forschung und bietet den Doktoranden Zugang zu High-End-Technologien und interdisziplinären Forschungsprojekten.

Das *Internationale Doktorandenprogramm* von MDC und Humboldt-Universität (HU) wurde 2003 ins Leben gerufen, um Nachwuchswissenschaftlern eine interdisziplinäre Ausbildung innerhalb eines strukturierten Rahmenprogramms zu bieten. Jedes Jahr gehen mehr als 1000 Bewerbungen ein, woraus etwa 100 Kandidatinnen und Kandidaten zu Bewerbungsgesprächen nach Berlin eingeladen werden. Von den 50-100 Doktoranden, die jährlich ihr Dissertationsprojekt in einer der Arbeitsgruppen am MDC oder dessen Partnern, der HU, der Freien Universität (FU), der Charité-Universitätsmedizin oder dem Leibniz-Institut für Molekulare Pharmakologie (FMP) aufnehmen, kommen mehr als 40% aus dem Ausland (verteilt auf 36 Länder).

Auf Grund des Erfolgs des *Internationalen Doktorandenprogramms* sprach die Helmholtz-Gemeinschaft dem MDC und seinen Universitätspartnern 2007 Fördermittel für die Gründung der *Helmholtz-Graduiertenschule Molecular Cell Biology (HGS-MCB)* und für die weitere Unterstützung der strukturierten, interdisziplinären Ausbildung von Doktoranden zu. Gegenwärtig gibt es am MDC etwa 350 Promovierende, die das vielseitige Ausbildungsprogramm der Graduiertenschule nutzen. Unter Berücksichtigung persönlicher Bedürfnisse und Interessen können die Doktoranden ihren individuellen Ausbildungsplan zusammenstellen. Die während der Promotion erbrachten Leistungen werden in einem Punktesystem dokumentiert und in einem Abschlusszertifikat zusammengefasst.

Die *HGS-MCB* bietet:
- Ein Welcome Center, das Promovierenden aus dem Ausland den Start in Berlin erleichtert
- Eine Schnittstelle zu den Universitäten
- Ein kollegiales Betreuungssystem: regelmäßige Treffen der Doktoranden mit einem Betreuungskomitee zur Diskussion des Projektfortschritts

- Annual Campus Symposium and PhD Retreat to promote networking and scientific exchange; both are co-organized by students
- Travel and collaboration funds to support attendance of international conferences, workshops, and visits in collaborating laboratories
- Building an alumni network

Four specialized research schools and international exchange programs have been established and are integrated within the *HGS-MCB.* These are smaller, thematically focused doctoral programs that provide tailored training and foster scientific networks within specific research areas at the MDC.

Helmholtz Research School "Molecular Neurobiology" (MolNeuro)

The close collaboration between the MDC and the FU led to the establishment of the *Helmholtz Research School in Molecular Neurobiology (MolNeuro)* in 2007. The curriculum focuses on basic and advanced concepts of Molecular Neurobiology. The Berlin Brain Days, a biannual conference for PhD students offer a Berlin-wide platform for young scientists to meet and discuss their research projects.

Helmholtz Research School „Translational Cardiovascular and Metabolic Medicine" (TransCard)

In 2009 the *TransCard Research School* was established as a collaborative endeavor between the MDC, the Charité, the HU, and the FU as well as the Hubrecht Laboratory in Utrecht, Netherlands, and the University of Arizona in Tucson AZ, USA as international partners. The school is embedded within the Cardiovascular and Metabolic Diseases Research Program, which covers a diverse spectrum of research areas, ranging from cellular biology and organ development, to genetics and pathophysiology. The program trains excellent young researchers at the interface of basic molecular, genetic, and clinical research with the goal to elucidate the molecular mechanisms causing cardiovascular and metabolic diseases and to transfer this knowledge from bench to bedside. The scientific curriculum consists of a lecture and seminar series, annual PhD retreats, E-learning units, and a clinical visits program in cooperation with the HELIOS Klinikum Berlin-Buch that provides students unique insight into the clinical perspective.

International Exchange Program in Systems Biology (MDC-NYU PhD Exchange Program)

In 2009, the Berlin Institute for Medical Systems Biology and the Center for Genomics and Systems Biology of the New York University started their joint *PhD Exchange Program.* The students are working on collaborative projects between two labs, bridging the research interests and technological approaches in various topics of gene regulatory elements and medical systems biology. All students are under supervision

- Wissenschaftliche Weiterbildung: Vorlesungen, Seminare, Methodenkurse, Einführung in neue Technologien, Seminare zur „Guten wissenschaftlichen Praxis"
- Professionelle Weiterbildung und Karriereplanung als Ergänzung zur wissenschaftlichen Ausbildung
- Reise- und Forschungsstipendien für die Teilnahme an internationalen Konferenzen und Methodenkursen oder Forschungsaufenthalten im Ausland
- Jährliches von den Doktoranden organisiertes Campus-Symposium und PhD-Retreat zur Förderung der Vernetzung und des wissenschaftlichen Austauschs
- Aufbau eines Alumni-Netzwerks

Seit 2007 wurden neben der Graduiertenschule kleinere, thematisch fokussierte Doktorandenprogramme gegründet und in die HGS-MCB integriert:

Helmholtz-Kolleg "Molecular Neurobiology" (MolNeuro)

Die enge Zusammenarbeit zwischen dem MDC und der FU führte 2007 zur Gründung des Helmholtz-Kollegs „Molecular Neurobiology" *(MolNeuro).* Im Fokus des Curriculums stehen Konzepte der Molekularen Neurobiologie (Grundlagen und spezialisierte Konzepte). Die alle zwei Jahre stattfindende Graduiertenkonferenz „Berlin Brain Days" bietet Doktoranden aus ganz Berlin die Möglichkeit zum Austausch und zur Diskussion ihrer Forschungsprojekte.

Helmholtz-Kolleg „Translational Cardiovascular and Metabolic Medicine" (TransCard)

Das 2009 gegründete Helmholtz-Kolleg *(TransCard)* ist eine Kooperation zwischen dem MDC, der Charité, der HU und der FU sowie dem Hubrecht Laboratory in Utrecht, Niederlande, und der University of Arizona in Tucson AZ, USA, als internationale Partner. Das Kolleg ist in das Programm Herz-Kreislauf- und Stoffwechselerkrankungen eingebettet und deckt somit ein breites Spektrum verschiedener Wissenschaftsgebiete von der Zell- und Entwicklungsbiologie, über Genetik bis hin zur Pathophysiologie ab. Das Programm bildet herausragende junge Wissenschaftler an der Schnittstelle von molekularer Grundlagenforschung und klinischer Forschung im Bereich translationaler Herz-Kreislauf- und Stoffwechselerkrankungen aus. Das wissenschaftliche Curriculum umfasst eine Vorlesungs- und Seminarreihe, jährliche Retreats, E-Learning-Einheiten sowie ein Clinical Visits-Programm in Kooperation mit dem HELIOS-Klinikum Berlin-Buch, das den Studenten hilft, ihre eigenen Arbeiten in einen translationalen Kontext einzuordnen.

MDC-NYU PhD Exchange Program

Das Berlin Institute for Medical Systems Biology (BIMSB) und das Center for Genomics and Systems Biology der New York University riefen 2009 ihr gemeinsames PhD-Austauschprogramm ins Leben. Die Doktoranden arbeiten an gemeinschaftlichen Projekten, die wissenschaftliches Interesse und technologische Fra-

of a MDC and a NYU faculty member and conduct their research in the respective laboratories, both in Berlin and New York.

German-Israeli Helmholtz Research School "Frontiers in Cell Signaling and Gene Regulation" (SignGene)

Established in 2013, *SignGene* is a joint endeavor between the MDC, the HU, and the Charité in Berlin, as well as the Technion, Israel Institute of Technology in Haifa, and the Hebrew University of Jerusalem. Research and training within *SignGene* focus on the areas of cellular signaling, the mechanisms of gene regulation, and quantitative biology with the goal to understand the molecular basis of diseases such as cancer. Within this interdisciplinary framework, *SignGene* students conduct their thesis projects in bilateral cooperation and benefit from the expertise of their main supervisor at the home institute and a co-mentor abroad. Prolonged exchange visits at the co-mentor's laboratory are integral to the curriculum, as are biannual training and networking events, which bring together scientists from the two countries.

gestellungen auf dem Gebiet der Genregulation und der medizinischen Systembiologie miteinander verbinden. Zusätzlich zu ihrer Betreuerin oder ihrem Betreuer am MDC haben alle Studierenden ein Fakultätsmitglied der New York University als Mentor und betreiben ihre Forschung in den entsprechenden Laboren sowohl in Berlin als auch in New York.

Deutsch-Israelisches Helmholtz-Kolleg "Frontiers in Cell Signaling and Gene Regulation" (SignGene)

Das 2013 gegründete deutsch-israelische Austauschprogramm *SignGene* ist eine Kooperation zwischen dem MDC, der HU und der Charité in Berlin sowie dem Technion, Israel Institute of Technology in Haifa und der Hebrew University in Jerusalem. Die Schwerpunkte des Forschungs- und Ausbildungsprograms liegen auf den Gebieten der zellulären Signalübertragung, den Mechanismen der Genregulation und der quantitativen Biologie mit dem Ziel, die molekularen Grundlagen der Krankheitsentstehung wie z.B. Krebs zu verstehen. In diesem interdisziplinären Rahmen arbeiten *SignGene*-Doktoranden an bilateralen Kooperationsprojekten und profitieren von der Expertise ihres Hauptbetreuers am Heimatinstitut sowie einem Co-Mentor im Ausland. Längere Forschungsaufenthalte im Partnerlabor sind integraler Bestandteil des Curriculums, ebenso wie zweimal pro Jahr stattfindende wissenschaftliche Veranstaltungen, die die Kollegmitglieder aus beiden Ländern zusammenführen.

Inka Gotthardt from the PhD office explaining the program of the MDC's Graduate School at the CareerDay.
Inka Gotthardt vom PhD-Büro erläutert beim CareerDay des MDC das Programm der Graduiertenschule.



Photo: Guido Rottmann/MDC

MDC Postdoctoral Program

There are more than 450 scientists and visiting researchers from more than 45 different countries pursuing their academic career at the MDC, and their contribution to the MDC research excellence is difficult to overestimate. Among them are quite a few fellowship-holders supported by the Helmholtz Association, the Alexander von Humboldt Foundation, the Marie Skłodowska-Curie actions of the EC, the Fulbright Scholar Program, etc.

At the MDC we view the postdoctoral period as an essential part of professional training of our scientists and support them in gaining valuable knowledge and complementary skills important in pursuing a broad range of future careers: in academia, industry, governmental and non-profit organizations as well as through entrepreneurship, both in Germany and abroad. Towards this goal we are developing a Training and Career Development (TCD) Framework and offer both flexible and tailored support through:

- Researchers' Training Program (which includes lecture series, seminars and courses, broad range of soft skills training offers, language and IT courses, etc.)
- Funding Opportunities and Support Service (via dedicated funding schemes and information events on funding opportunities, individual advice and grant writing support, etc.)
- Mentoring and Career Services (including a dedicated Mentoring program for female scientists, annual Career Day, "Career Pathways" talks, career counseling, etc.)
- Measures to ensure productive Work-Life Balance (e.g. Welcome Center taking care of all visa/work permit formalities, on-campus childcare facility, diverse sport activities, weekly "Beer-Hour" sessions).

Moreover, the MDC Postdoctoral Association promotes informal networking and stimulates closer interaction between postdoctoral scholars at the same career stage by organizing regular social events, monthly meetings over lunch, pizza evenings, joint excursions and theatre tours, etc.

Mehr als 450 Forscherinnen und Forscher sowie Gäste aus mehr als 45 Ländern verfolgen ihre akademische Karriere am MDC, und ihr Beitrag zur Exzellenz des MDC ist kaum zu überschätzen. Unter ihnen finden sich Stipendiaten der Helmholtz-Gemeinschaft, der Alexander-von-Humboldt-Stiftung, des Marie-Sklodowska-Curie-Programms der Europäischen Kommission und des Fulbright Scholar Programms.

Am MDC betrachten wir die Postdoc-Zeit als wesentlichen Bestandteil der beruflichen Ausbildung unserer Forschenden. Wir unterstützen sie bei der Erlangung von Wissen und ergänzenden Fähigkeiten für vielfältige zukünftige Karrierewege: in Wissenschaft, Industrie, bei staatlichen und Non-Profit-Organisationen sowie als Unternehmer, sowohl in Deutschland als auch im Ausland. Mit diesem Ziel haben wir ein Weiterbildungs- und Karriereentwicklungsprogramm entwickelt und bieten flexible und maßgeschneiderte Unterstützung durch:

- Weiterbildungsprogramm für Forscherinnen und Forscher (Vortragsreihen, Seminare und Kurse, eine breite Palette von Soft-Skills-Trainingsangeboten, Sprach- und EDV-Kurse)
- Fördermöglichkeiten und Support-Service (mit Hilfe spezieller Förderprogramme und Informationsveranstaltungen zu Finanzierungsmöglichkeiten, individueller Beratung und Unterstützung bei der Beantragung von Forschungsgeldern)
- Mentoring und Karriere-Services (einschließlich einem speziellen Mentoring-Programm für Wissenschaftlerinnen, dem jährlich stattfindenden Career Day, "Career Pathways"-Veranstaltungen und Karriereberatung)
- Maßnahmen zur Gewährleistung einer produktiven Work-Life-Balance (z. B. Übernahme der Formalitäten für Visum oder Arbeitserlaubnis durch das Welcome Center, Kindertagesstätte auf dem Campus, diverse Sportaktivitäten, informelle wöchentliche Treffen ("Beer-Hour").

Darüber hinaus fördert die MDC Postdoctoral Association die informelle Vernetzung. Für eine engere Zusammenarbeit zwischen Postdoktoranden in der gleichen Phase ihrer Karriere organisiert sie regelmäßige Veranstaltungen, monatliche Mittagstreffen, Pizza-Abende, Ausflüge sowie Theaterbesuche.

MDC International

The MDC is a truly international research institute: more than a third of its scientists come from more than 50 countries and almost a half of our PhD students are international. EU-citizens form the largest group of MDC researchers (with nationals of Italy, France and Spain topping the list). Chinese scientists form the largest single foreign-nationality community at the MDC, with more than 40 researchers and PhD students working and visiting the MDC at any time, and there are also large groups of nationals of India, Russia, USA, Brazil, Turkey, etc.

MDC researchers successfully cooperate with their peers around the globe: more than half of the MDC's publications (2012-2013) appeared in co-authorship with international partners from approximately 50 countries, with the USA, UK, and France leading the list. Renowned international scientists are frequently invited to the MDC to give a lecture or a seminar, for sabbaticals and collaborative visits. Several distinguished international scientists were awarded prestigious prizes to strengthen their cooperation with the MDC scientists: e.g. **Prof. Michael Glickman** from the Technion-Israel Institute of Technology received the **Friedrich Bessel Research Award of the Alexander von Humboldt Foundation** in 2012, and **Prof. Jehudit Bergman** from the Hebrew University in Jerusalem was honored with the **Helmholtz International Fellow Award** in 2013.

Jointly organized bilateral meetings provide an excellent platform to discuss the latest scientific findings and identify promising ideas for joint projects. In 2012-2013 seven such joint symposia and retreats took place both at the MDC and abroad with partners from Israel, France, China and the UK. The MDC also takes an active part in the recently established **Helmholtz-Israeli Cooperation in Personalized Medicine,** with the first tandem research projects to be selected for funding in early 2014. Fruitful cooperation of individual research group leaders often translates into closer collaborative links in form of joint research groups, e.g.:

- **The MDC-INSERM (Institut National de la Santé et de la Recherche Médicale) group** "Stem cell and macrophage biology" led by Dr Michael Sieweke at the Centre of Immunology Marseille-Luminy (CIML) (since 2012), which is part of the Helmholtz-INSERM Franco-German cooperation initiative;

Das MDC ist eine wahrhaft internationale Forschungseinrichtung: Mehr als ein Drittel der Forscherinnen und Forscher stammen aus über 50 Ländern und fast die Hälfte der PhD-Studenten sind aus dem Ausland. Angeführt von den Nationalitäten Italien, Frankreich und Spanien bilden EU-Bürger die größte Gruppe unter den Forschenden am MDC. Als einzelne Nationalität ragt China hervor: Mehr als 40 Forscher und PhD-Studenten oder Gastwissenschaftler kommen daher. Zusätzlich gibt es große Gruppen aus Indien, Russland, den USA, Brasilien und der Türkei.

Forscher des MDC kooperieren erfolgreich mit Kolleginnen und Kollegen aus aller Welt. Mehr als die Hälfte der Publikationen (2012 bis 2013) erschien in Koautorenschaft mit internationalen Partnern aus annähernd 50 Ländern, angeführt von den USA, Großbritannien und Frankreich. Das MDC lädt regelmäßig renommierte Wissenschaftlerinnen und Wissenschaftler ein, im Rahmen eines Sabbatjahres oder von Kooperationen Vorträge und Seminare zu geben. Um die Kooperationen mit MDC-Wissenschaftlern zu verstärken, haben etliche hervorragende internationale Forscher prestigeträchtige Preise erhalten: **Professor Michael Glickmann** vom Technion – Israel Institute of Technology erhielt beispielsweise 2012 den **Friedrich Wilhelm Bessel-Forschungspreis der Alexander von Humboldt-Stiftung** und **Professor Jehudit Bergman** von der Hebräischen Universität Jerusalem wurde 2013 mit dem **Helmholtz International Fellow Award** ausgezeichnet.

Gemeinsam organisierte bilaterale Veranstaltungen bilden exzellente Plattformen, um aktuelle wissenschaftlichen Erkenntnisse und vielversprechende Ideen für gemeinsame Projekte zu diskutieren. Sieben solcher gemeinsamer Symposien und Klausurtagungen fanden zwischen 2012 und 2013 am MDC oder im Ausland mit Partnern aus Israel, Frankreich, China und Großbritannien statt. Mit den ersten Tandem-Forschungsprojekten (die Auswahl soll Anfang 2014 erfolgen), übernimmt das MDC außerdem eine aktive Rolle in der kürzlich etablierten **Helmholtz-Israel-Kooperation in Personalisierter Medizin.** Erfolgreiche Kooperationen einzelner Forschungsgruppenleiter münden oft in enge Verbindungen, in Form gemeinsamer Forschungsgruppen:

- **Die MDC-INSERM (Institut National de la Santé et de la Recherche Médicale)-Gruppe** „Stammzellbiologie und Makrophagen". Sie wird seit 2012 von

- **Helmholtz-Russia Joint Research Group** "Possible beneficial effects of TNF and/or lymphotoxin ablation in carcinogen-induced and sporadic cancer, as studied in mice" (2011-2014), led by Dr. Marina Drutskaya (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow) in cooperation with Prof. Thomas Blankenstein at the MDC;
- **Helmholtz International Research Group** "Metabolism and Neurodegeneration" (2013-2016) led by Dr. Vanessa Schmidt and Prof. Thomas Willnow (both MDC) in cooperation with Dr. Mads Kjolby (MIND, Lundbeck Foundation, Aarhus University, Denmark);
- **Helmholtz International Research Group** "Role of Kinins in Obesity" (2013-2016) led by Prof. Michael Bader and Dr. Natalia Alenina (both MDC) in cooperation with Prof. João B. Pesquero (Universidade Federal de São Paulo, Brazil), co-funded by the São Paulo Research Foundation FAPESP.

Another important area of the MDC's activities, where international cooperation is of major importance, is the doctoral training of its PhD students. In addition to hosting international undergraduate students from North America (e.g. within the MIT-Germany, DAAD RISE and Fulbright programs) and Europe (e.g. within the EU Leonardo da Vinci Program SignGene), the MDC has established collaborative bilateral PhD programs with the USA, France and with Israel.

Working in an international context – through the supervision by two cooperating scientists from Germany and a cooperating country – and prolonged research stays abroad form an integral part of training and equips the next generation of young researchers with essential skills and international experience. In addition to improving the level of training and increasing the MDC's international visibility, these programs play a significant role in stimulating further collaborative links between MDC researchers and their international partners.



Photo: SignGene

The concept of the German-Israeli Helmholtz Research School "SignGene" was finalized in Haifa, Israel.
Das Konzept von SignGene wurde in Haifa diskutiert und verabschiedet.
From left to right (v.l.n.r.): SignGene Faculty Aaron Ciechanover (Technion, Nobel Prize Winner 2004), Amit Meller (Technion, SignGene speaker), Claus Scheidereit (MDC, SignGene Speaker), and Peretz Lavie, President of the Technion – Israel Institute of Technology.

Dr. Michael Sieweke vom Centre d'Immunologie de Marseille-Luminy (CIML) in Südfrankreich geleitet und ist Teil der französisch-deutschen Helmholtz-INSERM Kooperationsinitiative.

- **Helmholtz-Russia Joint Research Group:** In Kooperation mit Professor Blankenstein leitet Dr. Marina Drutskaya (Engelhardt Institut für Molekularbiologie, Russische Akademie der Wissenschaften in Moskau) die Forschungsgruppe „Die Rolle von TNF oder Lymphotoxin bei der Tumorentstehung" ("Possible beneficial effects of TNF and/or lymphotoxin ablation in carcinogen-induced and sporadic cancer, as studied in mice", 2011 bis 2014)
- **Helmholtz International Research Group:** Die internationale Helmholtz-Forschungsgruppe „Stoffwechsel und Neurodegeneration" (2013 bis 2016) leiten die MDC-Forscher Dr. Vanessa Schmidt und Professor Thomas Willnow in Kooperation mit Dr. Mads Kjolby vom MIND-Labor, Lundbeck Foundation an der Universität Aarhus in Dänemark.
- **Helmholtz International Research Group:** Die internationale Helmholtz-Forschungsgruppe „Rolle der Kinine bei Fettleibigkeit" (2013 bis 2016) leiten die MDC-Forscher Professor Michael Bader und Dr. Natalia Alenina in Kooperation mit Professor João B. Pesquero (Universidade Federal de São Paulo, Brazil), kofinanziert von der São Paulo Forschungs-Stiftung (Research Foundation, FAPESP).

Ein anderer wichtiger Bereich der Aktivitäten des MDC, bei denen internationale Kooperationen eine große Rolle spielen, ist die Ausbildung der PhD-Studentinnen und -Studenten. Zusätzlich zu der Betreuung internationaler grundständiger Studenten aus Nordamerika (beispielsweise über die Programme MIT-Germany, DAAD RISE und Fulbright Programs) und Europa (über das „Leonardo da Vinci" Programm) hat das MDC gemeinsame bilaterale PhD-Programme mit den USA, Frankreich und Israel etabliert.

Die Arbeit in einem internationalen Umfeld und die Betreuung durch zwei kooperierende Wissenschaftler aus Deutschland und dem Partnerland sowie ausgedehnte Forschungsaufenthalte im Ausland bilden einen wesentlichen Teil der Ausbildung und statten die nächste Generation junger Forscher mit wichtigen Fähigkeiten und internationaler Erfahrung aus. Zusätzlich zur Verbesserung des Ausbildungslevels und der internationalen Sichtbarkeit des MDC, spielen diese Programme eine wichtige Rolle dabei, Anreize für weitere Kooperationen zwischen MDC-Forschern und ihren internationalen Partnern zu schaffen.

Appointments

Berufungen

2012

Prof. Uwe Ohler appointed to W3-Professorship at Humboldt University Berlin

Uwe Ohler studied computational science at Friedrich Alexander University Erlangen where he also received his PhD. Then he worked as a postdoctoral fellow in the USA, first at the University of California at Berkeley, then at the Massachusetts Institute of Technology in Boston. Since 2005, Uwe Ohler was Assistant Professor at Duke University in Durham (USA). In this function, he made major contributions to the establishment of systems biology at Duke University. In September 2012, Uwe Ohler was appointed to a full professorship for Systems Biology of Gene Regulation at the Humboldt University. His research group is part of the MDC's Berlin Institute of Medical Systems Biology (BIMSB) and uses computational approaches to understand the biology of gene regulation in higher organisms.

BIMBS Junior Research Group for Dr. Baris Tursun

Baris Tursun began as a new junior group leader at MDC's BIMSB in February 2012. Dr. Tursun's team uses the roundworm *Caenorhabditis* elegans as a model organism. Their aim is to identify genes and epigenetic mechanisms that are involved in regulating reprogramming and direct cell fate conversions.

Dr. Tursun studied biology at the University of Hamburg. Then he joined Ingolf Bach's laboratory at the Center for Molecular Neurobiology (ZMNH), University of Hamburg, as a PhD student. In this time he was supported by a doctoral fellowship from the Boehringer Ingelheim Foundation. He completed his PhD and continued his work as a postdoctoral fellow until 2006. Baris Tursun then accepted a postdoctoral position in the laboratory of Oliver Hobert at Columbia University/Howard Hughes Medical Institute, New York. There he also received a "Francis-Goelet" postdoctoral fellowship, a prestigious stipend only awarded to highly promising scientists.

Prof. Uwe Ohler wird auf eine W3-Professur an die Humboldt-Universität zu Berlin berufen

Uwe Ohler studierte Informatik an der Friedrich-Alexander-Universität in Erlangen. Nach der Promotion arbeitete er als Postdoc in den USA, zunächst an der University of California in Berkeley, dann am Massachusetts Institute of Technology in Boston. Von 2005 bis 2012 war Ohler als Assistant Professor an der Duke University in Durham (USA) zentral am Aufbau der Systembiologie beteiligt. Im September 2012 wurde Uwe Ohler auf die W3-Professur für Systembiologie der Genregulation an die Humboldt-Universität zu Berlin berufen. Seine Forschungsgruppe ist am Berliner Institut für Medizinische Systembiologie (BIMSB) des MDC angesiedelt und nutzt computerbasierte Ansätze, um die Biologie der Genregulation in höheren Organismen zu verstehen.

BIMSB-Nachwuchsgruppe für Dr. Baris Tursun

Baris Tursun begann im Februar 2012 als neuer Nachwuchsgruppenleiter am BIMSB. Seine Gruppe verwendet den Fadenwurm *Caenorhabditis* elegans als Modellorganismus für ihre Untersuchungen. Ziel ist es, die Gene und epigenetischen Mechanismen zu identifizieren, die in die Regulation der Reprogrammierung von Zellen involviert sind und somit Einfluss auf das Schicksal der Zellen nehmen. Nach dem Studium der Biologie an der Universität Hamburg machte Tursun seine Doktorarbeit am Zentrum für Molekulare Neurobiologie (ZMNH) der Universität Hamburg. In dieser Zeit wurde er durch ein Doktorandenstipendium der Boehringer Ingelheim Stiftung unterstützt. Er blieb bis 2006 als Postdoc am ZMNH. Dann wechselte er auf eine Postdoc-Position im Labor von Oliver Hobert an der Columbia University/Howard Hughes Medical Institute, New York, USA. Hier wurde er mit dem angesehenen "Francis-Goelet" Postdoktoranden-Stipendium ausgezeichnet, welches nur an sehr vielversprechende Nachwuchswissenschaftler verliehen wird.

Dr. Michael Sieweke joined the MDC

The Sieweke group is based at the Centre of Immunology Marseille-Luminy (CIML) in southern France, and joined the MDC in July 2012 as part of the Helmholtz-INSERM Franco-German cooperation initiative. The group is co-funded by the MDC and INSERM (Institut National de la Santé et de la Recherche Médicale). The work of Dr. Sieweke's team is interfacing immunology and stem cell research. Stem cells can renew themselves and differentiate into many cell types. There are large hopes that stem cells may revolutionize future medicine. These hopes are based on the idea that these immature, still 'undecided' cells can be coaxed into making large numbers of any particular cell type of the body to serve a therapeutic purpose. Dr. Sieweke studied biochemistry at Eberhard Karls University Tübingen and earned his doctorate at the University of California at Berkeley, USA. Then he moved to the European Laboratory for Molecular Biology (EMBL), Heidelberg, where he stayed for 7 years, first as a postdoctoral fellow, then as junior faculty member. In 1999, Dr. Sieweke accepted a group leader position at the CIML.

Dr. Michael Sieweke schließt sich dem MDC an

Die Arbeitsgruppe von Michael Sieweke hat ihren Sitz am Centre d'Immunologie de Marseille-Luminy (CIML) in Südfrankreich. Sie schloss sich dem MDC im Juli 2012 als Teil der Helmholtz-INSERM-Initiative zur deutsch-französischen Zusammenarbeit an. Die Gruppe wird von MDC und INSERM (Institut National de la Santé et de la Recherche Médicale) kofinanziert. Dr. Siewekes Gruppe arbeitet an der Schnittstelle zwischen Immunologie und Stammzellforschung. Stammzellen können sich selbst erneuern und in viele verschiedene Zelltypen differenzieren. Es gibt große Hoffnungen, dass Stammzellen die zukünftige Medizin revolutionieren. Man hofft, dass die noch unreifen Stammzellen dazu gebracht werden können, große Mengen eines bestimmten Zelltyps zu produzieren, die dann für therapeutische Zwecke eingesetzt werden können. Dr. Sieweke studierte Biochemie an der Eberhard-Karls-Universität Tübingen und erhielt seinen Doktortitel von der University of California, Berkeley, USA. Dann wechselte er an das Europäische Labor für Molekularbiologie (EMBL) in Heidelberg. Dort arbeitete er die nächsten sieben Jahre, zunächst als Postdoc, dann als Nachwuchsgruppenleiter. 1999 nahm Dr. Sieweke eine Gruppenleiterposition am CIML an.

Bust of Max Delbrück (by Hans Scheib) in front of the MDC.C, the MDC's communications center.
Büste von Max Delbrück (Ausschnitt) vor dem Kongresszentrum des MDC (Bildhauer: Hans Scheib).



Photo: David Ausserhofer/MDC



Photo: David Ausserhofer/MDC

The opening ceremony in the foyer of the Max Rubner House.
Blick von oben in das Foyer des Max-Rubner-Hauses bei der Einweihung des Gebäudes.

2013

Prof. Ana Pombo recruited from the Imperial College London to BIMSB

With Ana Pombo, program head of the Molecular Sciences Section of the Institute of Clinical Sciences, Imperial College London, and MRC group leader at the MRC Clinical Sciences Centre in London, UK, the MDC succeeded in recruiting an excellent female scientist to BIMSB. The joint appointment to a full professorship for Translational Regulation and Gene Architecture by the MDC and Humboldt University Berlin is supported through additional funding from the Impulse and Cooperation Fund of the Helmholtz President. Prof. Pombo's work is characterized by a high level of innovation and powerfully combines imaging methods with molecular and biochemical approaches. She significantly contributed to the understanding of the relationship between the organization of the cellular nucleus and transcriptional regulation. For her research achievements, Ana Pombo has been honored by the renowned Feulgen Award. Ana Pombo studied biochemistry at the University of Lisbon in Portugal. She continued her academic career in Great Britain as a PhD student in the lab of Peter Cook. After her doctoral work she stayed as a postdoctoral fellow at the University of Oxford for two years. Since 2000, Prof. Pombo has directed her own independent research group at the core-funded MRC Institute, Clinical Sciences Centre. Since 2002 she was honorary lecturer for Cell Biology at the Imperial College London. In 2011, she became professor for Cell Biology.

Prof. Ana Pombo wechselt vom Imperial College London ans BIMSB

Mit Ana Pombo, Leiterin der Molecular Sciences Section des Institute of Clinical Sciences, Imperial College London, und MRC-Gruppenleiterin am MRC Clinical Sciences Centre in London, UK, konnte eine exzellente Wissenschaftlerin ans BIMSB rekrutiert werden. Die Berufung auf die W3-Professur für Transkriptionale Regulation und Genarchitektur, die gemeinsam mit der Humboldt-Universität zu Berlin erfolgte, wird mit zusätzlichen Fördermitteln aus dem Impuls- und Vernetzungsfonds des Helmholtz-Präsidenten unterstützt. Prof. Pombos Arbeiten sind sehr innovativ und verbinden erfolgreich bildgebende Verfahren mit molekularen und biochemischen Ansätzen. Sie haben wesentlich zum Verständnis der Beziehung zwischen der Organisation des Zellkerns und der Transkriptionsregulation beigetragen. Ana Pombo wurde für ihre wissenschaftlichen Leistungen mit dem renommierten Feulgen-Preis ausgezeichnet. Nach einer biochemischen Ausbildung an der Universität Lissabon in Portugal setzte Ana Pombo ihre Karriere in Großbritannien fort. Im Anschluss an ihre Doktorarbeit im Labor von Peter Cook und einer zweijährigen Postdoc-Phase an der Universität Oxford erhielt sie im Jahr 2000 bereits eine unabhängige MRC-Forschungsgruppe. Ab 2002 war Ana Pombo zunächst als Honorardozentin für Zellbiologie am Imperial College London tätig. 2011 wurde sie zur Professorin für Zellbiologie ernannt.

BIMBS Junior Research Group for Dr. Marina Chekulaeva

In January 2013, Marina Chekulaeva came from the Friedrich Miescher Institute for Biomedical Research

BIMSB-Nachwuchsgruppe für Dr. Marina Chekulaeva

Marina Chekulaeva wechselte im Januar 2013 vom Friedrich-Miescher-Institut für Biomedizinische For-

in Basel to the BIMSB where she built up a junior research group. Dr. Chekulaeva's team studies the molecular mechanisms that regulate mRNA translation, localization and stability and the role of non-coding RNAs in this process. Dr. Chekulaeva studied biochemistry at the State University of Moldova in Kishinev (Republic of Moldova) and became a graduate student at the Institute of Protein Research, Russian Academy of Sciences, in Pushchino. She did her doctoral work at the European Molecular Biology Laboratory (EMBL) in Heidelberg, where she continued to work for another year as a postdoc after completing her doctorate. In 2006, she went on as a postdoctoral fellow to the Friedrich Miescher Institute.

Dr. Robert Zinzen starts BIMSB Junior Research Group

Robert Zinzen from the EMBL in Heidelberg started as a junior group leader at the Berlin Institute for Medical Systems Biology in March 2013. A research focus of Dr. Zinzen is the investigation of the nervous system development in the fruit fly *Drosophila melanogaster.* Dr. Zinzen did his graduate studies at the University of Georgia in Athens, Georgia (USA) and received his PhD degree in Molecular and Cell Biology from the University of California at Berkeley. Since 2007, he was postdoctoral researcher and later staff scientist in Eileen Furlong's group at the EMBL, where he got additional training in genomics and epigenomics.

Prof. Oliver Daumke receives W3 professorship at the FU

The biochemist and protein crystallographer Oliver Daumke accepted an appointment to the full professorship for Structural Biology of Membrane Trafficking at the Free University Berlin. Oliver Daumke studied biology at the University of Freiburg and the University of Sussex, UK. He received his PhD degree from the University of Cologne and made his PhD thesis at the Max Planck Institute for Molecular Physiology in Dortmund under the supervision of Alfred Wittinghofer. After a postdoctoral period at the lab for Molecular Biology in Cambridge, UK, he returned as a Helmholtz junior group leader to the MDC. In 2010, Prof. Daumke became Junior Professor at the Institute for Medical Physics and Biophysics at Charité. He has received numerous awards, including the Bayer Early Excellence in Science Award in 2010, a scholarship from the Human Frontier Science Program, the Otto Hahn Medal of the Max Planck Society and the Klaus Liebrecht Prize of the University of Cologne for the best dissertation. He continues to work with his group at the MDC during his full professorship. They will further investigate the structure and function of proteins which bind to cellular membranes and lead to structural changes. These proteins play a crucial role in the development of several diseases such as cancer, diabetes or infections. In 2013, Oliver Daumke received a Consolidator Grant from the European Research Council (ERC).

schung in Basel an das MDC, um hier ihre neue Nachwuchsgruppe am Berliner Institut für Medizinische Systembiologie (BIMSB) aufzubauen. Die Gruppe von Dr. Chekulaeva untersucht die molekularen Mechanismen, die die Translation, Lokalisation und Stabilität der mRNA regulieren, sowie die Rolle von nicht-kodierenden RNAs bei diesem Prozess. Dr. Chekulaeva studierte Biochemie an der Staatlichen Universität von Chisinau (Moldavien) und machte ihre Diplomarbeit am Institut für Proteinforschung der Russischen Akademie der Wissenschaften in Pushchino. Ihre Doktorarbeit fertigte sie am Europäischen Labor für Molekularbiologie (EMBL) in Heidelberg an. Dort setzte sie ihre Forschungsarbeiten für ein weiteres Jahr als Postdoc fort. 2006 wechselte sie für eine zweite Postdoc-Phase an das Friedrich-Miescher-Institut.

Dr. Robert Zinzen startet BIMSB Nachwuchsgruppe

Robert Zinzen vom EMBL in Heidelberg startete im März 2013 als Nachwuchsgruppenleiter am BIMSB. Die Gruppe von Dr. Zinzen beschäftigt sich mit der Entwicklung des Nervensystems in der Fruchtfliege *Drosophila melanogaster.* Dr. Zinzen studierte an der University of Georgia in Athens, Georgia (USA), und promovierte im Fach Molekulare und Zellbiologie an der University of California, Berkeley. Seit 2007 arbeitete Robert Zinzen als Postdoc in Eileen Furlongs Gruppe am EMBL. Dort wurde er zudem in der Genomik und Epigenomik ausgebildet.

W3-Professur an der FU für Prof. Oliver Daumke

Der Biochemiker und Proteinkristallograph Oliver Daumke nahm den Ruf auf eine W3-Professur für Strukturbiologie des Membranverkehrs der Freien Universität (FU) Berlin an. Prof. Daumke studierte an der Universität Freiburg Biologie, ging als Austauschstudent an die Universität von Sussex in Brighton, England, und wechselte zur Promotion an die Universität zu Köln. Seine Doktorarbeit verfasste er am Max-Planck-Institut für molekulare Physiologie in Dortmund bei Alfred Wittinghofer. Nach einer Postdoc-Phase am Labor für Molekularbiologie in Cambridge, England, kam Prof. Daumke als Helmholtz-Nachwuchsforscher an das MDC. 2010 wurde er Juniorprofessor am Institut für Medizinische Physik und Biophysik an der Charité. Er erhielt bereits zahlreiche Auszeichnungen, darunter 2010 den Bayer Early Excellence in Science Award, ein Stipendium des Human Frontier Science Program, die Otto-Hahn-Medaille der Max-Planck-Gesellschaft und den Klaus-Liebrecht-Preis der Universität Köln für die beste Doktorarbeit. Prof. Daumke wird sein Labor am MDC weiterführen. Hier untersucht er Struktur und Funktion von Proteinen, die an zelluläre Membranen binden und diese verformen. Für die Entwicklung von zahlreichen Krankheiten wie Krebs und Diabetes oder bei Infektionen spielen diese Proteine eine zentrale Rolle. Ende 2013 erhielt er einen Consolidator Grant des Europäischen Forschungsrates (ERC).

Awards

Preise

2011

November / December

Michael BADER
Franz-Gross-Wissenschaftspreis
Deutsche Hochdruckliga (DHL)

Aaron CIECHANOVER
Humboldt Research Award / Humboldt-Forschungspreis
Alexander von Humboldt Foundation / Stiftung
(for a research period at the MDC / für einen Forschungsaufenthalt am MDC)

Thomas WILLNOW
Research Grant / Förderpreis
Alzheimer Forschung Initiative (AFI)

2012

Michael GLICKMAN,
Technion, Israel Institute of Technology, Haifa
Friedrich Wilhelm Bessel Research Award / Forschungspreis
Alexander von Humboldt Foundation / Stiftung
(for a research period at the MDC / für einen Forschungsaufenthalt am MDC)

Hua JING (MDC), Julia KASE (Charité)
Curt-Meyer-Gedächtnispreis
Berliner Krebsgesellschaft e.V.

Nikolaus RAJEWSKY
Gottfried Wilhelm Leibniz Prize 2012
Deutsche Forschungsgemeinschaft (DFG) / German Research Foundation

Martin SCHAEFER
[Research Group / Arbeitsgruppe: Miguel Andrade]
Participant at the 63th Lindau Nobel Laureate Meeting / Teilnehmer beim 63. Lindauer Nobelpreisträgertreffen

Thomas SOMMER (MDC) & Michael GLICKMAN (Technion, Israel Institute of Technology, Haifa)
Preis der Deutschen Technion-Gesellschaft
Deutsche Technion-Gesellschaft e.V.

2013

Soyoung LEE & Clemens SCHMITT
Johann-Georg-Zimmermann-Forschungspreis 2013/2014
Förderstiftung der Medizinischen Hochschule Hannover

James POULET
Paul-Ehrlich- und Ludwig-Darmstaedter-Nachwuchs-Preis
Paul-Ehrlich-Stiftung

Klaus RAJEWSKY
José Carreras Award
European Hematology Association

Klaus RAJEWSKY
STS/CCS Honorary Medal
Signal Transduction Society und Cell Communication and Signalling

Sandrine SANDER
[Research Group / Arbeitsgruppe: Klaus Rajewsky]
Curt-Meyer-Gedächtnispreis
Berliner Krebsgesellschaft

Kristin STOCK
[Research Group / Arbeitsgruppe: Helmut Kettenmann]
Nachwuchswissenschaftlerinnenpreis
Forschungsverbund Berlin-Brandenburg (FVB)

European Research Council (ERC) Grants

ERC Advanced Grant

BeyOND
Metabolic basis of neurodegenerative disease
Thomas Willnow (starts in 2014)

TRANSPOSOstress
Impact of stress-induced transposon activities on human disease
Zsuzsanna Izsvák

CYTOVOLION
Ion homeostasis and volume regulation of cells and organelles
Thomas J. Jentsch

EXTREMOPHILE MAMMAL
Molecular exploitation of an extremophile mammal
Gary Lewin

LYMPHOMA
Modeling lymphoma pathogenesis in mice – from basic mechanisms to pre-clinical models
Klaus Rajewsky

ERC Consolidator Grant

MitoShape
Structural basis of mitochondrial inner membrane shape and dynamics
Oliver Daumke

ERC Starting Grant

CardioSplice
A systems and targeted approach to alternative splicing in the developing and diseased heart: Translating basic cell biology to improved cardiac function
Michael Gotthardt

BrainStates
Brain states, synapses and behaviour
James Poulet

IsletVasc
Molecular Mechanisms Regulating Pancreatic Islet Vascularization
Matthew Poy

HEPATOPANCREATIC
Mechanisms underlying cell fate decision between pancreas and liver
Francesca Spagnoli

ThermoReg
Peripheral and Central Mechanisms of Temperature Detection and Core Body Thermoregulation
Jan-Erik Siemens (since February 2013 Medical Faculty of Heidelberg)

Third-party Funding 2012-2013

Drittmittelprojekte

Selected projects (ausgewählte Projekte)

EU-Funding

Collaboration

Health

EUROSPIN
European Consortium on Synaptic Protein Networks in Neurological and Psychiatric Diseases

EURATRANS
European large-scale functional genomics in the rat for translational research

SynSys
Synaptic Systems: dissecting brain function in health and disease

Information and Communication Technologies

TARGETAMD
Transposon-based, targeted ex vivo gene therapy to treat age-related macular degeneration

3x3D Imaging
Fast two-photon in vivo imaging and stimulation with simultaneous three-dimensional random-access scanning in multiple brain regions

Nanotechnologies

MARINA
Managing Risks of Nanoparticles

Marie Curie

Career Integration Grant

DYNASTIIC
Dynamic Signal Transduction in Individual Cells

REPROL53U48
Characterization of direct reprogramming-regulating factors LIN-53 and USP-48

WNT/CALCIUM IN HEART
WNT/Calcium signaling in cardiovascular development

Industry-Academia Partnerships and Pathways

InduStem
Comparative stem cell research in mouse and humans

Intra-European Fellowship

Atherochemokine
Investigation of the role of CXCL5/CXCR1 pathway in atherosclerosis

Olig3-Lbx1 breathing
Function of the transcription factors Olig3 and Lbx1 in brainstem respiratory nuclei

PostScribeDNAdamage
Global characterization of post-transcriptional regulatory interactions in DNA damage response

International Incoming Fellowship

RNARegMap
Condition specific RNA Regulatory Maps

International Outgoing Fellowship

Kinase crosslinking
Capturing kinase-substrate pairs in intact mammalian cells by using unnatural amino acid mutagenesis and photocrosslinking

International Reintegration Grant

ENDOPANC
Novel signals guiding endodermal progenitor cells toward a pancreatic fate

MuRF and hypertrophy
Regulation and function of the E3 ubiquitin ligases Muscle RING finger 1 and 3 in cardiac hypertrophy

Initial Training Network

EVONET
Evolution of gene regulatory networks in animal develpoment

ATTRACT
Advanced Teaching and Training for Adoptive Cell Therapy

Infrastructures

EU-OPENSCREEN
European Infrastructure of Open Screening Platforms for Chemical Biology

ISBE
Infrastructure for Systems Biology - Europe

DFG Projects

Research Grants / Sachbeihilfe

A finite-element model of human phonation with fluid-structure interaction

Alternatives Spleißen in der perinatalen Herz-Entwicklung und bei Kardiomyopathie

Analyse der spezifischen Funktion paraloger alpha-Importine der Säuger

Charakterisierung der Funktion membranständiger Ubiquitin-Ligasen bei der Dislokation fehlerhafter Proteine

Critical contribution of proneurotrophin-receptor Sortilin to the pathology of multiple sclerosis and experimental autoimmune encephalomyelitis

Der EDA-A1/EDAR/NF-kappaB Signalweg als multifunktioneller Regulator der Haarfollikelinduktion

Diabetes, abdominal obesity, weight change, metabolic factors and risk of liver cancer in the European Prospective Investigation into Cancer and Nutrition

Die Bedeutung von Cytochrom P450 (CYP) 2J2 und deren Metabolite in der Pathogenese der Präeklampsie

Function of panglial gap junctional networks in mouse brain

Genetic regulation of CD177 and the role of CD177-proteinase-3 interactions in ANCA-associated vasculitis.

Genetics of endocardial-myocardial interactions during zebrafish heart development

Haarzyklusregulation durch NF-KB bei Mensch und Maus: Manipulation eines epithelialen Schlüsselsignals als innovativer Ansatz zur Therapie von Haarerkrankungen

In vivo tracking of manipulated dendritic cells in tumor models

Investigating the interaction of high-grade gliomas and microglial cells

Investigations on the roles of Claudins during zebrafish cardiovascular development

JIMI – a network for intravital mikroscopy: dedicated project supervision from cell imaging to cell tracking in living organisms

Mechanismen der Protein-Qualitätskontrolle des endoplasmatischen Retikulums

Mechanisms of DNA damage induced IKK and NF-kB activation

Mechanisms of the switch of stem cells to cancer stem cells in head and neck cancer

Molecular causes of primary angle-closure glaucoma

Molecular characterization of cis- and trans-acting gene and ncRNA regulation during human chondrogenesis

Molekulare Charakterisierung cis- und trans-aktiver Gen- und ncRNA-Regulation während der humanen Chondrogenese

Post translational modifications (PTM) of C/EBPß and functional implications

Shaping a lymphoma survival niche within secondary lymphoid organs

Simulation of excitation contraction coupling in ventricular cardiac myocytes

Sorting-Rezeptoren als Regulatoren des Lipoproteinstoffwechsels und des kardiovaskulären Risikos

Structural and functional analyses of panglial coupling networks and its impact on brain signalling.

Structural Studies of the Nucleocapsids of Negative-strand RNA Viruses as Targets of the Dynamin-like Myxovirus Resistance Protein A

Strukturelle und funktionelle Charakterisierung von ß-Amyloid Aggregations-Intermediaten

Targeting the secretory pathway in cytotoxic T lymphocytes to modulate their cytolytic capacity in cancer immunotherapy

The (pro)renin receptor and cardiovascular disease

The Coxsackievirus and Adenovirus Receptor CAR in Cardiac Remodeling

The role of NF-kB in an animal model of ANCA-associated vasculitis and therapeutic options using NF-kB inhibition

Titin in Biomechanik, Signaltransduktion und Stoffwechsel Funktionelle Analyse der N2B-Region im Vergleich der Geschlechter

Research Units / Forschergruppen

FOR 667:
Epithelial Mechanisms in Renal Volume Regulation

FOR 748:
Neuronal and glial P2 receptors; molecular basis and functional significance

FOR 806:
Interfering with intracellular protein-protein interactions - probing protein functions with small molecules

FOR 1054:
Sex-Specific Mechanisms in Myocardial Hypertrophy

FOR 1341:
Barrel Cortical Function

FOR 1352:
Structure, Function and Regulation of the Myofibrillar Z-disc Interactome

FOR 1368:
Hemodynamic Mechanisms of Acute Kidney Injury

Clinical Research Units / Klinische Forschergruppen

KFO 192:
Regulation und Fehlregulation von Muskelwachstum

KFO 218:
Hormonal regulation of body weight maintenance

Research Fellowships / Forschungsstipendien

Dissection and optogenetic manipulation of the Habenula-IPN cell-specific neuronal networks in the control of pain and addiction

The molecular mechanism of Semaphorin 3E-Plexin-D1 signaling in shaping vascular topology during development

Priority Programmes / Schwerpunktprogramme

SPP 1395:
Information and Communication Theory in Molecular Biology

SPP 1356:
Pluripotency and Cellular Reprogramming

SPP 1365:
The regulatory and functional network of ubiquitin family proteins

SPP 1395:
Informations- und Kommunikationstheorie in der Molekularbiologie (InKoMBio)

SPP 1230:
Mechanisms of gene vector entry and persistence

SPP 1463:
Epigenetic regulation of normal hematopoiesis and its dysregulation in myeloid neoplasia

Independent Junior Research Groups / Emmy Noether-Nachwuchsgruppen

Die Rolle ß-Catenin/TCF-abhängiger transkriptioneller Netzwerke bei Morphogenese und Regeneration renaler Epithelien

Collaborative Research Centres / Sonderforschungsbereiche

SFB 618:
Theoretical Biology: Robustness, modularity and evolutionary design of living systems

SFB 633:
Induction and Modulation of T-Cell Mediated Immune Responses in the Gastrointestinal Tract

SFB 650:
Cellular Approaches for the Suppression of Unwanted Immune Reactions - From Bench to Bedside

SFB 665:
Developmental Disturbances in the Nervous System

SFB 740:
Von Molekülen zu Modulen: Organisation und Dynamik zellulärer Funktionseinheiten

SFB 958:
Scaffolding of Membranes: Molecular Mechanisms and Cellular Functions

Research Training Groups / Graduiertenkollegs

GRK 1772:
Computational Systems Biology

CRC / Transregios

TRR 19:
Inflammatory Cardiomyopathy - Molecular Pathogenesis and Therapy

TRR 36:
Principles and Applications of Adoptive T Cell Therapy

TRR 43:
The brain as a target of inflammatory processes

TRR 52:
Transcriptional Programming of Individual T Cell Subsets

Clusters of Excellence / Exzellenzcluster

EXC 257:
NeuroCure – towards a better outcome of neurological disorders

Graduate Schools / Graduiertenschulen

GSC 86:
Berlin School of Mind and Brain

GSC 203:
Berlin-Brandenburg School for Regenerative Therapies

GSC 1091:
Berlin School of Integrative Oncology (BSIO)

International Research Training Groups / Internationale Graduiertenkollegs

GRK 1631:
International Research Training Group for Myology

Conferences and Scientific Meetings

Kongresse und wissenschaftliche Tagungen

2012

Date / Datum	Event / Veranstaltung	Organizer / Veranstalter
30.03.2012	**Green Campus – Lebendige Labore Nachhaltige Wissenschaft**	MDC, Abt. TFM Errichten
19.04.–20.04.2012	**9. Laborrunde – Konferenz**	MDC, Abt. TFM Errichten
03.05.–04.05.2012	**SFB Meeting: 2nd Symposium on Adoptive T Cell Therapy**	Prof. Th. Blankenstein Prof. W. Uckert
09.05.–12.05.2012	**International Spring Meeting Force Transduction & Emerging Ion Channels**	Prof. G. Lewin
10.05.2012	**Auftaktveranstaltung Deutsches Zentrum für Herz-Kreislauf-Forschung**	Dr. J. Krebser, DZHK
08.06.2012	**3rd Annual Scientific Symposium „Ultrahigh Field Magnetic Resonance: Clinical Needs, Research Promises and Technical Solutions**	Prof. Th. Niendorf Dr. B. Ittermann Prof. J. Schulz-Menger
28.06.–30.06.2012	**5th Berlin Summer Meeting „Computational & Experimental Molecular Biology“**	Prof. N. Rajewsky Prof. K. Rajewsky
07.09.–08.09.2012	**Franz Volhard Symposium zu Ehren des 70. Geburtstages von Prof. Luft**	Prof. D. Müller
25.10.2012	**German French Symposium Frontiers of Cardiovascular Research**	Dr. O. Seumenicht

2013

Date / Datum	Event / Veranstaltung	Organizer / Veranstalter
25.04.–26.04.2013	**10. Laborrunde – Konferenz**	MDC, Abt. TFM Errichten
23.04.–24.05.2013	**Brain Tumor Meeting**	Prof. H. Kettenmann
31.05.2013	**4th Annual Scientific Symposium „Ultrahigh Field Magnetic Resonance Clinical Need, Research Promises, Technical Solutions"**	Prof. T. Niendorf
13.06.–15.06.2013	**6th Berlin Summer Meeting Computational & Experimental Molecular Biology**	Prof. N. Rajewsky Prof. K. Rajewsky
13.06.–15.06.2013	**Deutsch-Chinesisches Kardio- Meeting**	Prof. M. Bader
13.09.–14.09.2013	**60 Jahre DNA,Kongress für Lehrkräfte, Studierende, Schüler und Interessierte**	Dr. L. Bengtsson Abt. Kommunikation
10.11.–13.11.2013	**Stem Cells in Development and Disease**	Dr. D. Besser, GSCN

Seminars 2012-2013

2012

First name	Last name	Organization	Talk title
David R.	**Borchelt**	University of Florida, Dept. of Neuroscience, Gainsville	Why can't I remember
Tewis	**Bouwmeester**	Novartis Institutes for Biomedical Research, Basel	Drugging the Wnt pathway
Reinhard	**Büttner**	Institute of Pathology, Cologne	LSD1 and epigenetics in cancer
Bruce	**Carter**	Vanderbilt University, Nashville	Chromatin remodelling during Schwann cell differentiation
Raymond	**Deshaies**	Howard Hughes Med. Institute, Pasadena	Regulation of the assembly and function of cullin-RING ubiquitin ligases
Stefanie	**Dimmeler**	Goethe University, Institute of Cardiovascular Regeneration, Frankfurt	MicroRNAs: therapeutic targets and diagnostics in cardiovascular disease
Ildiko Rita	**Dunay**	Otto-von-Guericke-Universität Magdeburg, Institut für Medizinische Mikrobiologie	Role of myeloid cell subsets in parasitic infections
Diego	**Echevarria**	University Miguel Hernandes, Alicante	Reading FGF8 morphogenetic activity during neural tube patterning
Anastasia	**Eskova**	BIOQUANT, Heidelberg	High content analysis of integrin alpha2 endocytosis regulators
Laura	**Forero**	King's College London	A novel role for neurotransmitters in sensory organ and forebrain development
Maxim	**Frank-Kamenetskii**	Boston University	DNA reactivity with formaldehyde and the double helix's breathing modes
Shigeyoshi	**Fujiwara**	National Research Institute for Child Health and Development, Tokyo	Humanized mouse models of Epstein-Barr virus infection and associated diseases
Matthias	**Futschik**	Centre for Molecular and Structural Biomedicine, University of Algarve	
Patricia	**Gaspar**	Institut du Fer à Moulin, INSERM U839, Paris	Deciphering the variety of serotonin subsystems
Toby	**Gibson**	EMBL, Heidelberg	Diversity of Protein-Protein Interactions, Modular Protein Architecture, and the Nature of Cell Regulation
Christine	**Hartmann**	Institute of Molecular Pathology, Vienna	WNTing embryonic stem cells
Michael T.	**Heneka**	Klinik und Poliklinik für Neurologie, Klinische Neurowissenschaften, Bonn	Multiple level interactions between neuroinflammation and neurodegeneration in Alzheimer´s disease

First name	Last name	Organization	Talk title
Manuel	**Hessenberger**	Paris Lodron University, Salzburg	The crystallization and characterization of NLRP14_PYD
Kie	**Kasuga**	Karolinska Institutet Stockholm, Sweden	"A Pipeline for MHC-II Ligand Identification from a Single Patient"
Ziad	**Khattari**	Hashemite University, Zarqua	Viral ion channel proteins in model membranes: a comparative study by X-ray reflectivity
Stefan	**Knapp**	Nuffield Department of Clinical Medicine at the University of Oxford	Selective targeting of protein interactions mediated by acetyl lysine reader domains
Marlies	**Knipper**	Hörforschungszentrum der Univerität Tübingen	Molecular Basis of Tinnitus
Florian	**Kohlhepp**	Dept. for Infectious Diseases, Heidelberg	EHD proteins in Apicomplexa parasites
Marina	**Kolesnichenko**	The Scripps Research Institute, La Jolla	Attenuation of TORC1 suppresses replicative and RAS-induced cellular senescence
Robert S.	**Krauss**	Mount Sinai School of Medicine, New York	Modeling Holoprosencephaly in the Mouse: Regulation of Sonic Hedgehog Signaling by Co-receptors
Young-Ae	**Lee**	ECRC / Charité / Max Delbrück Center (MDC), Berlin	Molecular dissection of inflammation in allergic diseases - Towards novel interventional targets
Eleonora	**Leucci**	Biotech Research and Innovation Centre (BRIC), University of Copenhagen	Role of miR-9 in RNA metabolism and Lymphomagenesis
Elena A.	**Levashina**	Max-Planck-Institut für Infektionsbiologie, Berlin	Mosquito immune responses against malaria parasites
Steve	**Ley**	MRC National Institute for Medical Research, London	Regulation of inflammation by the MAP kinase kinase kinase TPL-2
Bettina	**Linnartz**	Institut für Rekonstruktive Neurobiologie, Universität Bonn	Sialic acid residues as triggers of complement receptor-3 dependent removal by microglia?
Michal	**Malewicz**	MRC Toxicology Unit, Leicester	Direct regulation of DNA repair by NR4A nuclear orphan receptors
Matthias	**Mann**	Max Planck Institute of Biochemistry, Munich	Application of proteomics to large scale protein sequencing and genetics
Matthew	**Movsesian**	University of Utah School of Medicine, Salt Lake City	Phophoselective PDE3 inhibition: a new therapeutic strategy for heart failure?
Sabine	**Müller**	Ernst-Moritz-Arndt-Universität, Greifswald	Playing with RNA conformation: Hairpin ribozyme variants for RNA repair, self-processing and ligand sensing
Boris	**Musset**	Rush University Medical Center, Chicago	The selectivity of Hv1 and proton and electron currents in human monocytes
Daniel	**Otzen**	Aarhus University, Dep. of Molecular Biology and Genetics	The role of the oligomer in alpha-synuclein aggregation and Parkinson's Disease
Evangelia	**Petsalaki**	Mount Sinai Hospital, Toronto	Building the Rho-ome: An integrative study of the role of RhoGAP/GEFs in the Rho protein signaling network
Britta	**Qualmann**	Friedrich-Schiller-University Jena Institute of Biochemistry	The impact of membrane remodelling on neuromorphogenesis, vesicle formation and neuronal network activity
Tobias	**Reichenbach**	The Rockefeller University, New York	Biophysics of hearing
Anne	**Ridley**	King's College London	Rho GTPase signaling in cell adhesion and migration

First name	Last name	Organization	Talk title
Maria	**Sakkou**	Max-Planck Institute of Neurobiology, Martinsried	EphB2 trans-endocytosis during contact repulsion is mediated by Src/Dock180/Elmo signaling
Brenda	**Schulman**	St. Jude Children`s Res. Hospital, Memphis	Twists and turns in ubiquitin conjugation cascades
Almut	**Schulze**	Cancer Research UK London Research Institute	Regulation of glucose and lipid metabolism by oncogenic signalling pathways in cancer
John E.	**Shively**	Beckman Research Institute Of City of Hope, Duarte	The role of CEACAM1 in inflammation and cancer
Umberto	**Spampinato**	Université Victor Segalen Bordeaux 2 Centre Recherche Inserm U862 - Institut Francois Magendie 146	Role of central 5-HT2B receptors in the control of dopamine ascending
Anje	**Sporbert**	Max Delbrück Center (MDC), Microscopy Core Facility, Berlin	Basic principles and potential applications of superresolution microscopy. Part 2: Localisation microscopy methods (PALM, STORM, GSDM)
Christine	**Stadelmann-Nessler**	Institut für Neuropathologie, Göttingen	Grey matter damage and repair in inflammatory demyelination
Eduard	**Stefan**	University Innsbruck	Novel insights into evolutionary conserved protein kinase A signalling circuits
Esta	**Sterneck**	National Cancer Institute, Faro	C/EBPdelta: Dr. Jekyll and Mr. Hyde in breast cancer biology
Shiro	**Suetsugu**	Institute of Molecular and Cellular Biosciences, Tokyo	Acute regulation of cell surface area by the balance between flattening and endocytosis of caveolae
Juliana	**Tavares**	Department of Physiology and Biophysics, Belo Horizonte	Cerebral leukocyte recruitment: an essential crosstalk between immune and vascular system
Aaron	**Voigt**	Universität Aachen	Functional genetics using Drosophila models of human neurodegenerative diseases
Yanli	**Wang**	Institute of Biophysics Beijing	Structural Insight into RNA Induced Gene Silencing
Andreas	**Werdich**	Harvard Medical School, Boston	Cardiac electrical remodelling triggered by stretch: what can we learn from the zebrafish?
Cornelia M.	**Weyand**	Stanford School of Medicine, Division of Immunology and Rheumatology	T cell effector mechanisms in atherosclerosis
Andrea	**Wizenmann**	Institute of Anatomy, Tübingen	Guidance of retinal ganglion cells by Engrailed
Michael	**Yaffe**	MIT, Boston	Michael Yaffe (MIT): Post-translational modification of proteins, PPIs, and cellular signaling

2013

First name	Last name	Organization	Talk title
Jeroen	**Bakkers**	Hubrecht Institute, Utrecht	Studying cardiac development using zebrafish genetics
Veronique	**Baud**	Institut Cochin- INSERM, Paris	New insights into RelB function and regulation
Tom	**Becker**	Brain and Mind Research Institute, Sydney	Human Genomics in transgenic Zebrafish
Axel	**Behrens**	London Research Institute, Cancer Research UK	Stem cells, cell fate reprogramming, and cancer
Heinz-Georg	**Belting**	Biozentrum, University of Basel, Dept. Cell Biology	Action at the junction: Distinct roles for VE-cadherin during vascular network formation
Rochelle	**Buffenstein**	Barshop Institute for Aging, Dept. of Physiology, Texas	The role of the proteasome and resistasome in sustained good health in the long lived naked mole rat
Monica	**Campillos**	Helmholtz Center, Munich	From phenotypic information to molecular information
Ramu	**Chenna**	TU Dresden	New Gap Treatments in Biological Sequence Alignment and a Tool for Exploring Functional Context of Protein Motifs
Zoltan	**Cseresnyes**	Deutsches Rheumaforschungszentrum Berlin (DRFZ) and Max Delbrück Center (MDC), Berlin	Striped-illumination deep-infrared multiphoton microscopy: a novel tool for high-resolution deep-tissue imaging at the MDC
Hans-Ulrich	**Dodt**	Department of Bioelectronics Institute of Solid State Electronics TU Vienna	3D-Ultramicroscopy of whole mouse brains and mouse embryos
Phillip	**Drewe**	Computational Biology Center, Sloan-Kettering Institute, New York	Accurate detection of differential RNA processing
Simon	**Ebbinghaus**	Ruhr-Universität Bochum	Protein folding and aggregation kinetics probed in the cell
Sandra	**Fernandez Moya**	Institute of Parasitology and Biomedicine, Granada, Spain	"Functional characterization of RNA binding proteins DRBD3 and RBP33 in Trypanosoma brucei"
Nir	**Friedman**	Hebrew University of Jerusalem	Systematic dissection of roles for chromatin regulators in a yeast stress response
Olga	**Garaschuk**	Physiologisches Institut, Lehrstuhl II, Eberhard Karls Universität Tübingen	High resolution in vivo imaging of microglia
Rosemarie	**Grantyn**	Exzellenzcluster NeuroCure, Synaptic Dysfunction Group and Experimental Neurology, Berliln	Neural circuit repair by deep brain stimulation
Ernesto	**Guccione**	Institute of Molecular and Cell Biology (IMCB), Singapore	Splicing regulator PRMT5 links Alternative Splicing to the p53 response in mammalian development
Matthias	**Günther**	Fraunhofer Mevis, Bremen	Noninvasive perfusion measurement: basics and not-so basics of arterial spin labeling
Julia	**Haseleu**	Max Delbrück Center (MDC), Berlin	Seeing deeper: tissue clearing methods applied in thick-sample imaging
Kristina	**Heinig**	Max Delbrück Center (MDC), Berlin	Mechanismen der Entstehung einer Überlebensnische für murine CLL-Leukämiezellen in sekundären lymphatischen Organen

First name	Last name	Organization	Talk title
Laurence	**Hurst**	University of Bath, Dept. of Biology and Biochemistry, Somerset	Why does selection care about codon usage (or whatg really determines ribosome velocity)?
Kazuhiro	**Iwai**	Kyoto University	Linear polyubiquitination: a new regulator of NF-kappaB activation
Wanchana	**Jangsangthong**	University of Cologne	Structural determinants of gating properties of voltage-gated L-type Ca2+ channel as revealed by single-channel analysis
Andreas	**Jenny**	Albert Einstein College of Medicine, New York	Canonical and non-canonical Wnt signaling in Drosophila
Batsheva	**Kerem**	The Hebrew University of Jerusalem, Life Sciences Institute	The molecular basis for chromosomal instability in early stages of cancer development
Michael	**Kirschbaum**	Fraunhofer Institute for Biomedical Engineering, Potsdam	Spatio-temporal control of cell adhesion and neurite outgrowth via microstructured thermoresponsive polymer coatings
David	**Komander**	MRC Laboratory of Molecular Biology, Cambridge	Mechanisms, roles, and uses of OTU domain deubiquitinases
Ralf	**Kühn**	Helmholtz-Zentrum München	Direct production of mouse mutants using ZFNs, TALENs or Crispr/Cas in one-cell embryos
Limor	**Landsman**	Dept. of Cell and Developmental Biology,Sackler Faculty of Medicine, Tel Aviv	Pancreas development and function: the role of mesenchymal cells
Ralf	**Langen**	University of Southern California, Los Angeles	How proteins bend membranes
Han	**Li**	Spanish National Cancer Centre, Madrid	Tumor Suppressor Genes in the biology of Stem Cells
Calum	**MacRae**	Harvard Medical School, Brigham and Women's Hospital, Boston	Systems approaches in cardiac and vascular development
Harmit	**Malik**	Fred Hutchinson Cancer Research Center, Seattle	Genetic conflicts: the usual suspects and beyond
Yael	**Mandel-Gutfreund**	Technion - Israel Institute of Technology, Haifa	Identifying Protein Recognition Elements on RNA by Combining Sequence and Structural Information
Sascha	**Martens**	Max-Perutz-Laboratories, University of Vienna	Molecular Mechanisms of Autophagosome Formation: Insights from Reconstitutions
Rajeswari R.	**Moganty**	All India Institute of Medical Sciences, New Delhi	Role Of DNA Triple Helical Structures In Neurodegenerative Disorders, Friedreich's Ataxia
Martin	**Morad**	Cardiac Signaling Center, Medical University of South Carolina, Clemson	Calcium Signaling in human inducted pluripotent stem cell-derived Cardiomyocytes from normal and Catecholaminergic polymorphic ventricular tachycardia-afflicted subjects
Christian	**Mosimann**	University of Zürich	Tales of stripes: deciphering cell fates in the lateral plate and the neural crest
Yaakov	**Nahmias**	Hebrew University, Jerusalem	Introduction to Microfluidics: Applications in Biology
Teresa	**Nicolson**	Howard Hughes Medical Institute, Chevy Chase, Maryland	Ribbon synapses in hair cells: size matters
Raluca	**Niesner**	Deutsches Rheumaforschungszentrum Berlin (DRFZ)	The state of JIMI: ongoing intravital imaging projects in the MDC-DRFZ network

First name	Last name	Organization	Talk title
Laura	**Oehme**	Deutsches Rheumaforschungszentrum Berlin (DRFZ)	Statistical methods applied in the analysis of in-vivo imaging data
Alejandro	**Panjkovich**	Institute of Biotechnology and Biomedicine, Universitat Autónoma de Barcelona	Protein allosteric sites - a structural bioinformatics perspective
Nathalie	**Rouach**	Neuroglial Interactions in Cerebral Physiopathology, Collège de France, Paris	Unraveling the many ways astroglial connexins control neurotransmission
Maike	**Sander**	University of California San Diego	Gene regulatory networks controlling pancreatic beta cell development
Jannike	**Sarmadi**	Deutsches Rheumaforschungszentrum Berlin (DRFZ)	Automated cell tracking in intravital imaging: limitations and advantages
Eyal	**Scheijter**	Weizmann Institute of Science, Rehovot, Israel	Actin Cables Guide Polarized Secretion in Tubular Organs
Andrea	**Schietinger**	University of Washington, Seattle	Molecular mechanisms of T cell tolerance to self/tumor antigens
Karin	**Schlegelmilch**	Boston Children's Hospital	YAP1 and the Hippo Signaling Pathway Regulate Progenitor Proliferation
Alexey	**Semyanov**	RIKEN Brain Science Institute (BSI), Hirosawa	Mechanisms regulating subcellular Ca2+ dynamics in astrocytes
Robert H.	**Shoemaker**	National Cancer Institute, Bethesda	Real-time kinetic analysis of the in vitro invasion phenotype of the NCI60 tumor cell line panel enables identification of genes associated with mesenchymal invasion and inhibitory compounds.
Konstantinia	**Skreka**	Biocenter/Medical University Innsbruck	''Identification and expression profiling of novel ncRNAsinvolved in mESC in vitro neural differentiation''
Anje	**Sporbert**	Max Delbrück Center (MDC), Berlin	The state of JIMI: ongoing intravital imaging projects in the MDC-DRFZ network
Florian	**Stengel**	ETH Zurich	Mass Spectrometry supported determination of protein complex structure – an integrated approach
Francis	**Stewart**	BIOTEC TU Dresden	Gastrulation in the mouse depends upon a switch between histone 3 lysine 4 methyltransferases
Franz	**Theuring**	Charite - Universitätsmedizin Berlin CCR/ Institut für Pharmakologie	Treating Alzheimer's Disease by using Tau-Aggregation-Inhibitors: the time for Tau is NOW
Sakari	**Vanharanta**	Sloan-Kettering Institute, New York	Deconstructing Metastasis - Epigenetic Expansion of the VHL-HIF Signal Output Drives Renal Cancer Progression
Alf	**Wachsmann**	Max Delbrück Center (MDC), Berlin	eduroam – wireless network everywhere
Hua	**Yu**	City of Hope National Medical Center, Duarte, USA	Novel approaches to target STAT3 in specific immune subsets: opportunities for clinical use
Niccolo	**Zampieri**	Columbia University, New York	Position, recognition, and the assembly of motor circuits

Annex

Anhang



Helmholtz Association

Helmholtz-Gemeinschaft

The Helmholtz Association is the largest science organization in Germany with its approximately 36,000 employees and collaborators in 18 research centers as well as with an annual budget of about 3.8 billion Euro, ca. 30% of which are third-party funds from the public and private sector.

The research centers of the Helmholtz Association are active in six fields of research: (1) Energy, (2) Earth and Environment, (3) Health, (4) Key Technologies, (5) Structure of Matter as well as (6) Aeronautics, Space and Transport. Within these research fields, thematic programs are defined that are supported by one or several centers. Scientists from the respectively involved centers jointly develop the research program in which they work together in a cross-center approach. This structure of program-oriented funding (POF) was introduced in 2001 following a suggestion by the German Council of Science and Humanities.

The research field Health focuses on the major widespread diseases. Research is being carried out in five programs: (I) cancer research, (II) cardiovascular and metabolic diseases, (III) nervous system disorders, (IV) infection and (V) genetic and environmental influences on widespread diseases with a focus on diabetes mellitus, chronic lung diseases and allergies. The MDC coordinates the program "Cardiovascular and Metabolic Diseases" and substantially contributes to the programs "Cancer Research" and "Functions and Dysfunctions of the Nervous System".

The most important research activities in the field of health research are concentrated at five centers: at the German Cancer Research Centre (DKFZ) in Heidelberg, at the German Center for Neurodegenerative Diseases (DZNE) in Bonn, which was newly established in 2009, at the Helmholtz Zentrum München – German Research Center for Environmental Health (HMGU), at the Helmholtz Centre for Infection Research (HZI) in Braunschweig and at the Max Delbrück Center for Molecular Medicine (MDC) Berlin Buch. Moreover, other Helmholtz centers make vital contributions: the Helmholtz-Zentrum Dresden-Rossendorf (HZDR), the GSI Helmholtz Centre for Heavy Ion Research in Darmstadt and the Helmholtz Centre for Environmental Research (UFZ) in Leipzig.

When it comes to topics of increasing significance, such as personalized medicine, collaboration takes place

Die Helmholtz-Gemeinschaft ist mit ihren rund 36.000 Mitarbeiterinnen und Mitarbeitern in 18 Forschungszentren sowie einem Jahresbudget von rund 3,8 Milliarden Euro, davon ca. 30% Drittmittel aus dem öffentlichen und privatwirtschaftlichen Bereich, die größte Wissenschaftsorganisation Deutschlands.

Die Forschungszentren der Helmholtz-Gemeinschaft arbeiten in sechs Forschungsbereichen: (1) Energie, (2) Erde und Umwelt, (3) Gesundheit, (4) Schlüsseltechnologien, (5) Struktur der Materie sowie (6) Verkehr und Weltraum. Innerhalb dieser Forschungsbereiche sind thematische Programme definiert, die von einem oder mehreren Zentren getragen werden. Wissenschaftler aus den jeweils beteiligten Zentren entwickeln gemeinsam das Forschungsprogramm, in denen sie Zentrenübergreifend zusammenarbeiten. Diese Struktur der programmorientierten Forschung (POF) wurde 2001 auf Vorschlag des Wissenschaftsrates eingeführt.

Der Forschungsbereich Gesundheit konzentriert sich auf die großen Volkskrankheiten. Die Forschung erfolgt in fünf Programmen: (I) Krebsforschung, (II) Herz-Kreislauf- und Stoffwechselerkrankungen, (III) Erkrankungen des Nervensystems, (IV) Infektion und (V) Gen-Umwelt-Einflüsse auf Volkskrankheiten mit Schwerpunkt auf Diabetes mellitus, chronischen Lungenerkrankungen und Allergien. Das MDC koordiniert das Programm „Herz-Kreislauf- und Stoffwechselerkrankungen" und beteiligt sich substantiell an den Programmen „Krebsforschung" und „Erkrankungen des Nervensystems".

Die wichtigsten Forschungsaktivitäten im Gesundheitsbereich sind an fünf Zentren angesiedelt: am Deutschen Krebsforschungszentrum (DKFZ) in Heidelberg, am Deutschen Zentrum für Neurodegenerative Erkrankungen (DZNE) in Bonn, das 2009 neu gegründet wurde, am Helmholtz Zentrum München für Gesundheit und Umwelt (HMGU), am Helmholtz-Zentrum für Infektionsforschung (HZI) in Braunschweig und am Max-Delbrück-Centrum für Molekulare Medizin (MDC). Darüber hinaus leisten weitere Helmholtz-Zentren wichtige Beitrage: Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Helmholtz-Zentrum für Schwerionenforschung (GSI) in Darmstadt, und Helmholtz-Zentrum für Umweltforschung (UFZ) in Leipzig.

Bei Themen von zunehmender Bedeutung, wie der personalisierten Medizin, wird Zentren- und Programm-



Photo: Heidi Scherm/Helmholtz-Gemeinschaft

A team of five researchers led by Klaus Butterbach-Bahl (2nd from right) of the Karlsruhe Institute of Technology (KIT) was awarded the "Wissenschaftspreis des Stifterverbandes – Erwin Schrödinger-Preis 2013". This prize is jointly awarded by the Stifterverband für die Deutsche Wissenschaft and the Helmholtz Association.
Ein fünfköpfiges Forscherteam um Klaus Butterbach-Bahl (2.v.r.) vom Karlsruher Institut für Technologie (KIT) wurde bei der Helmholtz-Jahrestagung 2013 mit dem Wissenschaftspreis des Stifterverbandes – Erwin Schrödinger-Preis 2013 ausgezeichnet. Den Preis vergeben der Stifterverband für die Deutsche Wissenschaft und die Helmholtz-Gemeinschaft jedes Jahr gemeinsam.

across centers and programs. To this end, partners are invited, especially from the field of university medicine. For strengthening translational research, the Helmholtz Association has established partnerships with universities, in particular the field of university medicine, other extramural research institutions and the industry. These partnerships are being continuously further developed. Moreover, the health research centers of the Helmholtz Association are partners of the German Centres for Health Research and of the German National Cohort.

Program-oriented funding POF

In autumn 2001, the entire research of the Helmholtz Association was newly structured. The Helmholtz Association bundled its research activities within the six major research fields mentioned above and defined thematic programs within these research fields. Resources are not allocated to individual institutions, but rather to cross-center research programs, which compete with each other.

A strategic review, carried out every five years, provides the basis for funding these research programs. This task is carried out by well-known experts from across the globe. Their assessments provide the basis for the decision to which amount the federal government and the federal states provide funding for the individual programs.

The first funding period ran from 2003 to 2008, the second program period covered the time between 2009/2010 and 2013/2014, depending on the time of assessment. The assessments for the second funding period took place in spring 2008 (Aeronautics, Space

übergreifend zusammengearbeitet. Dazu werden Partner insbesondere aus der Hochschulmedizin eingebunden. Zur Stärkung der translationalen Forschung hat die Helmholtz-Gemeinschaft Partnerschaften mit Universitäten, insbesondere der Hochschulmedizin, weiteren außeruniversitären Forschungsinstitutionen sowie mit der Industrie etabliert. Diese werden kontinuierlich weiter entwickelt. Darüber hinaus sind die Gesundheitsforschungszentren der Helmholtz-Gemeinschaft Partner der Deutschen Zentren der Gesundheitsforschung sowie der Nationalen Kohorte.

Die Programmorientierte Förderung POF

Im Herbst 2001 wurde die gesamte Forschung der Helmholtz-Gemeinschaft neu strukturiert. Die Helmholtz-Gemeinschaft hat ihre Forschungsaktivitäten in den sechs oben genannten großen Bereichen gebündelt und innerhalb dieser Forschungsbereiche thematische Programme definiert. Ressourcen werden nicht einzelnen Institutionen zuerkannt, sondern Zentrenübergreifenden Forschungsprogrammen, die sich untereinander im Wettbewerb befinden.

Eine strategische Begutachtung, die alle fünf Jahre stattfindet, bildet die Basis für die Finanzierung der Forschungsprogramme. Diese Aufgabe übernehmen renommierte Experten aus aller Welt. Ihre Gutachten bilden die Grundlage für die Entscheidung, in welcher Höhe Bund und Länder die einzelnen Programme fördern.

Die erste Förderperiode lief von 2003 bis 2008, die zweite Programmperiode von 2009/2010 bis 2013/2014, je nach Zeitpunkt der Begutachtung. Die Begutachtungen für die zweite Förderperiode erfolgten im Frühjahr 2008 (Verkehr und Weltraum, Ge-

and Transport, Health, Earth and Environment) and in spring 2009 (Energy, Structure of Matter, Key Technologies). The program assessments for the third funding period took place in spring 2013 for the research fields Aeronautics, Space and Transport, Health, Earth and Environment and are scheduled for spring 2014 for the research fields Energy, Structure of Matter and Key Technologies.

In both a national and international comparison, the reviewers appraised the scientific quality of all programs in the research field Health to be outstanding and of extraordinary strategic relevance for society, the economy and science.

In addition to the research programs, the Helmholtz centers maintain large-scale research infrastructures that are used by scientists both at home and abroad. These infrastructures are subject to a separate review process. With its involvement in the German National Cohort, the research field Health for the first time establishes a large-scale infrastructure. The participation of the Helmholtz centers in the German National Cohort was assessed separately.

Assessment of centers

The MDC performed successfully in all three program reviews carried out in the context of the third funding period in March 2013 and received the highest marks from the reviewers for its scientific achievements. The results of these assessments impressively confirm the result of the center review that took place in March 2012.

Between the reviews in the context of program-oriented funding, assessments of the individual centers take place on the level of the research groups. The research groups at the MDC were last reviewed in March 2012. A total of 38 external reviewers assessed the work of the research groups at the MDC directly on site. All in all, the MDC was assessed to be "highly visible on an international level" and received the highest mark of "outstanding" since more than a third of all research groups were given the highest mark. Of the 62 research groups, 29% received the highest mark "outstanding", 26% were rated "outstanding to excellent" and 26% were rated "excellent." This means that a total of 81% of research groups were rated to contribute excellent and outstanding scientific achievements. Moreover, the reviewers highlighted the specific strategic orientation of the MDC with its cross-disease approach as a mark of strength and basis for success.

sundheit, Erde und Umwelt) und im Frühjahr 2009 (Energie, Struktur der Materie, Schlüsseltechnologien). Die Programmbegutachtungen für die dritte Förderperiode erfolgten für die Forschungsbereiche Verkehr und Weltraum, Gesundheit, Erde und Umwelt im Frühjahr 2013 und stehen für die Forschungsbereiche Energie, Struktur der Materie, Schlüsseltechnologien im Frühjahr 2014 an.

Im nationalen und internationalen Vergleich schätzten die Gutachter die wissenschaftliche Qualität aller Programme im Forschungsbereich Gesundheit als herausragend und von außerordentlicher strategischer Relevanz für die Gesellschaft, Wirtschaft und Wissenschaft ein.

Neben den Forschungsprogrammen betreiben die Helmholtz-Zentren große Forschungsinfrastrukturen, die national und international genutzt werden. Diese werden einer separaten Begutachtung unterzogen. Der Forschungsbereich Gesundheit etabliert mit seiner Beteiligung an der Nationalen Kohorte erstmalig eine große Infrastruktur. Der Anteil der Helmholtz-Zentren an der Nationalen Kohorte wurde einer separaten Begutachtung unterzogen.

Begutachtung der Zentren

Das MDC hat in allen drei Programmbegutachtungen im Rahmen der dritten Förderperiode im März 2013 erfolgreich abgeschnitten und Höchstnoten von den Gutachtern für seine wissenschaftlichen Leistungen erhalten. Die Ergebnisse dieser Begutachtungen bestätigten eindrucksvoll das Ergebnis der Zentrumsbegutachtung vom März 2012.

Zwischen den Begutachtungen im Rahmen der programmorientierten Förderung finden die Begutachtungen der einzelnen Zentren auf der Ebene der Forschungsgruppen statt. Die Forschungsgruppen des MDC wurden zuletzt im März 2012 begutachtet. Insgesamt bewerteten 38 externe Gutachter vor Ort die Arbeit der Forschungsgruppen des MDC. Das MDC wurde insgesamt als „highly visible on an international level" und mit der Höchstnote „outstanding" bewertet, da mehr als ein Drittel aller Forschungsgruppen mit der Höchstnote bewertet wurden. Von den 62 Forschungsgruppen erhielten 29% die Höchstnote „outstanding, 26% „outstanding to excellent" und 26% „excellent". Damit wurden insgesamt 81% der Forschungsgruppen exzellente und herausragende wissenschaftliche Leistungen bescheinigt. Darüber hinaus haben die Gutachter die besondere Ausrichtung des MDC mit seiner krankheitsübergreifenden Aufstellung als Stärke und Basis für den Erfolg herausgestellt.

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The Helmholtz Association comprises 18 research centers in Germany.
Die Helmholtz-Gemeinschaft umfasst 18 Forschungszentren in Deutschland.

Berlin Institute of Health (BIH)

Berliner Institut für Gesundheitsforschung

In the Berlin Institute of Health (BIH) the Max Delbrück Center for Molecular Medicine (MDC) and the Charité – Universitätsmedizin Berlin join forces to promote translational research. Within the overarching theme of systems medicine the goal is to accelerate application of research findings into clinical practice (bench to bedside) and to increasingly address unmet medical needs in basic research (bedside to bench). In an interdisciplinary systemic approach the BIH aims at developing innovative concepts in diagnosis, therapy and prevention for the ultimate benefit of patients and improved public health.

Founded in the beginning of 2013 as a cooperation of MDC and Charité, the BIH is scheduled for early 2015 to become a corporation under public law (Körperschaft öffentlichen Rechts). This will lift the long-standing project-based collaboration of MDC and Charité to an institutional level and create a novel structure for translational research. Both partners, MDC and Charité, will keep their status as legal entities and thus retain their full independence. Responsibilities for fundamental affairs of each institution, such as research in the framework of the Helmholtz Association at the MDC, will not be affected.

Until 2018 the BIH can draw on a total budget of about 280 million Euros for its set-up. In 2013 and 2014 the designated volume of 38 million Euros will be provided by the Helmholtz Association. Thereafter the BIH will receive institutional funding by the Federal Government and the State of Berlin in a ratio of 90:10. By 2018 the institute will run on an annual budget of about 80 million Euros. Moreover, the BIH will be supported by the Private Excellence Initiative (Private Exzellenzinitiative) of Johanna Quandt who provides another 40 million Euros over the next ten years. The funds of the Private Excellence Initiative are managed by the Charité foundation (Stiftung Charité).

The institute will be operated by a four-member Executive Board: The Chair of the MDC, Walter Rosenthal, the Chair of the directorial board of the Charité, Karl Max Einhäupl, the Dean of the Charité, Annette Grüters-Kieslich, and an independent Chairman, Ernst Th. Rietschel. A Supervisory Board with members from the State of Berlin, the Federal Government, the Helmholtz Association and the Berlin Universities oversees the BIH. A Scientific Advisory Board consisting of 12

Im Berliner Institut für Gesundheitsforschung/Berlin Institute of Health (BIH) bündeln das Max-Delbrück-Centrum für Molekulare Medizin (MDC) und die Charité - Universitätsmedizin Berlin ihre Kräfte. Unter dem übergreifenden Forschungsansatz der Systemmedizin wird am BIH translationale Forschung betrieben: Erkenntnisse aus der Grundlagenforschung sollen zielgerichteter in die klinische Anwendung gebracht und klinische Fragestellungen verstärkt ins Labor getragen werden. Ziel ist es, durch interdisziplinäre und systemische Ansätze innovative Konzepte der Diagnostik, Therapie und Prävention zu entwickeln und diese zur Verbesserung der Gesundheit der Menschen umzusetzen.

Das BIH wurde Anfang 2013 als Kooperation des MDC und der Charité gegründet. Bis 2015 soll es per Landesgesetz in eine Körperschaft des öffentlichen Rechts überführt werden. Damit wird die langjährige projektbasierte Kooperation von MDC und Charité auf eine institutionelle Ebene gebracht und eine neue Struktur der Translationsforschung geschaffen. MDC und Charité als Gliedkörperschaften des BIH bleiben rechtlich selbstständig; sie können über eigene Belange, am MDC etwa die Forschung im Rahmenprogramm der Helmholtz-Gemeinschaft, weiter frei bestimmen.

Für den Aufbau des BIH stehen bis 2018 rund 280 Millionen Euro zur Verfügung. 2013 und 2014 stammen die vorgesehenen 38 Millionen Euro Förderung aus Mitteln der Helmholtz-Gemeinschaft. Danach erhält das BIH eine institutionelle Förderung durch den Bund und das Land in einem 90:10-Schlüssel. In der Endausbauphase in 2018 wird das BIH über einen Jahresetat von rund 80 Millionen Euro verfügen. Zusätzlich wird das BIH durch die „Private Exzellenzinitiative“ von Johanna Quandt gefördert, die das Institut über die nächsten zehn Jahre mit 40 Millionen Euro unterstützt. Diese Gelder werden von der Stiftung Charité verwaltet.

Dem BIH steht ein vierköpfiger Vorstand vor: der Vorstandsvorsitzende des MDC, Walter Rosenthal; der Vorstandsvorsitzende der Charité, Karl Max Einhäupl; die Dekanin der Charité, Annette Grüters-Kieslich; und ein unabhängiger Vorstandsvorsitzender, Ernst Th. Rietschel. Ein Aufsichtsrat mit Vertretern von Land, Bund, Helmholtz-Gemeinschaft und Berliner Universitäten beaufsichtigt das BIH. Die inhaltliche und strategische Begleitung des BIH erfolgt durch einen Beirat aus zwölf



Photo: David Ausserhofer/MDC

external and internationally recognized scientists advises and supports the BIH in scientific and strategic questions.

At the BIH, basic biomedical research, clinical research and medical practice are consolidated under one roof. Systems medicine lies at the heart of the BIH research program: Recent technological breakthroughs such as high-throughput technologies and powerful *omics* tools (Genomics, Proteomics, Metabolomics) have enabled the comprehensive molecular characterization of diseases. Combined with advances in bioinformatics and clinical data management this allows systems-oriented approaches to be applied to medicine and makes the holistic analysis of complex (patho-)physiological processes and their interplay possible. Instead of looking at single organs or pathologies, systems medicine focuses on overarching aspects of disease. The BIH will thus primarily address cross-cutting topics pertaining to multiple diseases such as immunology, cellular and sub-cellular processes, cell degeneration and regeneration, metabolism, the genetics of disease and gender medicine.

The complementary strengths of MDC and Charité make them ideal partners to pursue systems medicine-guided translational research. The MDC contributes its expertise in molecular and systems biology and provides access to animal models of human disease and state-of-the-art *omics* technologies. The Charité adds essential knowledge of disease mechanisms and in clinical phenotyping. It provides access to a large number of well-characterized patients over the whole range of common and rare diseases and has expert knowledge in the management of clinical trials, biobanks and clinical databases.

The BIH will be active in five areas: It will support excellent research in translational systems medicine, re-

externen international anerkannten Wissenschaftlerinnen und Wissenschaftlern.

Im BIH werden biomedizinische Grundlagenforschung, klinische Forschung und ärztliche Praxis unter einem Dach zusammengeführt. Im Mittelpunkt des Forschungsprogramms steht dabei die Systemmedizin: Die Entwicklung von Hochdurchsatzverfahren und leistungsfähigen *Omics*-Technologien (Genomics, Proteomics, Metabolomics) hat eine umfassende molekulare Charakterisierung von Krankheiten ermöglicht. Zusammen mit Fortschritten in der Bioinformatik und der klinischen Datenverarbeitung erlaubt das die Anwendung systemorientierter Ansätze in der Medizin, bei der komplexe (patho-)physiologische Prozesse und deren dynamische Wechselwirkungen in ihrer Gesamtheit untersucht werden. Statt um einzelne Organe oder spezielle Pathologien geht es um die Aufklärung übergreifender Aspekte von Krankheiten. Am BIH werden daher verstärkt Querschnittsthemen wie Immunologie, zelluläre und subzelluläre Prozesse, Degeneration und Regeneration von Zellen, Stoffwechselvorgänge, die Genetik von Krankheiten und die Bedeutung von Geschlechtsunterschieden in Krankheitsprozessen adressiert.

Für diesen Forschungsansatz bilden MDC und Charité mit ihren komplementären Stärken ideale Ausgangspartner. Das MDC bringt seine herausragende molekulare und systembiologische Expertise ein und bietet Zugang zu einer Vielzahl von Tiermodellen der Humanpathologie und zu neuesten Entwicklungen in den *Omics*-Technologien. Die Charité verfügt über große Expertise im Bereich der Krankheitsmechanismen und der klinischen Phänotypisierung. Sie ermöglicht den Zugang zu einer großen Anzahl von gut charakterisierten Patienten im ganzen Spektrum der häufigen bis seltenen Erkrankungen und verfügt über Expertenwissen



Photo: David Ausserhofer/BIH

At the opening ceremony of the BIH: Neuroscientist Alan Richardson-Klavehn accompanied a slide show on the piano. Background: The BIH Board of Directors Walter Rosenthal, Ernst Rietschel, Karl Max Einhäupl and Annette Grüters-Kieslich.
Bei der Eröffnungszeremonie des Berliner Instituts für Gesundheitsforschung begleitete der Neurowissenschaftler Alan Richardson-Klavehn eine Diashow am Piano. Im Hintergrund zu sehen ist der BIH-Vorstand mit Walter Rosenthal, Ernst Rietschel, Karl Max Einhäupl und Annette Grüters-Kieslich.

cruit outstanding scientists, provide cutting-edge technologies and infrastructures, build up *Clinical Research Units* and develop new career paths for early-career scientists.

To foster interdisciplinary collaboration of basic scientists and clinicians and to advance translational and systems medicine, competitive BIH programs provide funding for excellent research projects. *BIH Collaborative Research Grants* will finance large interdisciplinary consortia of MDC and Charité scientists at the interface of basic research and clinical practice. The first projects have started in spring 2014. *BIH Twinning Research Grants* will provide financial support for smaller collaborations.

Recruitment of complementary expertise in areas such as stem cell biology and bioinformatics is crucial to the scientific strategy and excellence of the BIH. To this end the BIH will create well-funded and internationally competitive *BIH Chair* positions. Additionally, the institute will provide supporting funding for appointbei klinischen Studien und der Arbeit mit Biobanken und klinischen Datenbanken.

Das BIH wird in fünf Bereichen aktiv. Es wird exzellente systemmedizinisch-translationale Forschung fördern, herausragende Wissenschaftler rekrutieren, hochmoderne Infrastrukturen und Technologie-Plattformen zur Verfügung stellen, *Clinical Research Units* aufbauen und neue Wege in der Nachwuchsförderung beschreiten.

Um die interdisziplinäre Zusammenarbeit von Grundlagenwissenschaftlern und klinischen Forschern zu fördern und im Sinne der Translation und Systemmedizin weiter auszubauen, fördert das BIH im Rahmen von kompetitiven Programmen exzellente Forschungsprojekte. *BIH Collaborative Research Grants* finanzieren von Wissenschaftlern und Klinikern aus MDC und Charité gemeinsam getragene, langfristig angelegte Verbundprojekte. Die erste Förderrunde ist im Frühjahr 2014 gestartet. *BIH Twinning Research Grants* unterstützen kleinere Kollaborationen.

ments at Charité or MDC with particular relevance to the BIH research program thus attracting top scientists to Charité and MDC.

Access to state-of-the-art technology platforms and infrastructures build the foundation for internationally competitive research at the BIH. This includes service for *omics* technologies with a common service platform mainly set up at the MDC site. Bioinformatics expertise for systemic analysis of molecular and clinical data will be consolidated in the *BIH Center of Bioinformatics* and supported by extension of IT infrastructures. Joint biobank structures will provide access to patient samples and imaging capacities for clinical phenotyping will be expanded. Measures for advancing stem cell biology in the BIH will include the set-up of a service unit for induced pluripotent stem cells. In addition, a unit for transgenic techniques will be established. Cooperation with the FMP secures access to state-of-the-art technologies for high-throughput screening of small molecule libraries.

To create an excellent framework for patient-oriented research and clinical studies, *Clinical Research Units* (CRU) will be established. As units independent of regular patient care they offer top infrastructure for investigator-initiated trials with patients and probands. Following the example of the ECRC at the campus Buch, which already comprises a functional CRU, the BIH will create further CRU at other Charité sites.

Educational activities of the BIH include stipends for medical students, programs for the qualification of *Clinical Scientists* and funding opportunities for doctoral and postdoctoral students with a special interest in clinical research. The different educational programs will be consolidated under the umbrella of the *BIH Academy of Biomedical Education and Training.* The BIH Academy aims at interlinking career support and training in systems and translational research. It will coordinate and establish training opportunities for undergraduate students, doctoral students, postdoctoral fellows and clinical researchers.

Thus, the BIH will train a new generation of "translational" scientists that bridge the gap between clinicals and experimental research.

Entscheidend für die inhaltliche Ausrichtung und Exzellenz des BIH wird die strategische Rekrutierung ergänzender Expertise sein, etwa in den Gebieten Stammzellbiologie und Bioinformatik. Das BIH schafft dafür kompetitiv ausgestattete *BIH Chair*-Positionen. Um die besten Köpfe zu gewinnen, bietet das BIH attraktive Zusatzausstattungen für an beiden Partnereinrichtungen vorgesehene Berufungen mit besonderer Relevanz für das BIH.

Der Zugang zu modernsten Infrastruktureinrichtungen und Technologie-Plattformen bildet die Basis für eine international kompetitive Forschung am BIH. Dazu gehören die *Omics*-Technologien, die als zentrale Plattform vor allem am Standort MDC angesiedelt sein werden. Die bioinformatische Expertise zur Auswertung der systemmedizinischen und klinischen Daten wird im *BIH Center of Bioinformatics* vereint und durch IT-Infrastrukturmaßnahmen unterstützt. Gemeinsame Biobanken ermöglichen den Zugang zu Patientenproben. Für die klinische Phänotypisierung werden die Imaging-Plattformen ausgebaut. Ein weiteres Standbein wird der Ausbau der Stammzellbiologie inklusive einer Service-Einheit für induzierte pluripotente Stammzellen sein. Zudem wird eine Einheit für transgene Techniken etabliert. Über eine Kooperation mit dem FMP wird der Zugang zu neuesten Techniken im Wirkstoff-Screening sichergestellt.

Um am BIH hervorragende Rahmenbedingungen für die patientenorientierte Forschung und für klinische Studien zu schaffen, werden *Clinical Research Units* (CRU) etabliert: Das sind vom regulären ambulanten und stationären Klinikbereich getrennte Einheiten, in denen Patienten und Probanden im Rahmen von wissenschaftsinitiierten Studien außerhalb der Regelversorgung behandelt werden können. Nach Vorbild des ECRC am Campus Buch, das bereits eine funktionsfähige CRU umfasst, werden im BIH weitere CRU-Strukturen an Standorten der Charité aufgebaut.

Zu den Ausbildungsaktivitäten des BIH gehören Stipendien für Studierende der Medizin, Programme für die Ausbildung von *Clinical Scientists,* und die Förderung von Doktoranden und Postdoktoranden mit einem besonderen Interesse an klinischer Forschung. Die Ausbildungsprogramme werden unter dem Dach der *BIH Academy of Biomedical Education and Training* vereint. Ziele der BIH Academy sind eine am gemeinsamen Forschungsparadigma orientierte Vernetzung der Nachwuchsförderung und die Schaffung und Koordination entsprechender Angebote für Studierende, Doktoranden, Postdoktoranden und forschende Kliniker.

Damit wird das BIH eine neue Generation von „translationalen" Wissenschaftlern ausbilden, die die Brücke zwischen Klinik und experimenteller Forschung bilden.

German Centre for Cardiovascular Research

Deutsches Zentrum für Herz-Kreislauf-Forschung

The Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK – German Centre for Cardiovascular Research) is one of six German health research centers and was established in 2011 by the German Federal Ministry of Education and Research. The DZHK has stepped up to bring together in a network cardiovascular researchers from the fields of basic and clinical research from all over Germany in order to create improved conditions for the translation of research results into clinical application practice.

The MDC is one of 27 member institutions of the DZHK and provides 9 principal investigators (PIs). Together with the Charité – Universitätsmedizin Berlin, the Deutsches Herzzentrum Berlin (DHZB, German Heart Institute Berlin) and the Robert Koch Institute, it constitutes the DZHK partner site "Berlin", one of seven such partner sites.

In the context of the DZHK, the MDC PIs are involved in larger site projects in the fields of vascular diseases, hereditary and inflammatory cardiac diseases, cardiac insufficiency, prevention and imaging. In addition, they conduct research together with other DZHK partners in cross-site cooperative projects.

Within the DZHK, MDC PIs and other MDC scientists offer their know-how and expertise in the form of so-called "shared expertise". By means of this shared expertise, the DZHK promotes synergies and collaboration between the respective sites. The two most used shared expertise fields at the DZHK (each used nine times) are "Generation and cardiovascular phenotyping of transgenic rats" and "Genomics/Proteomics" and are offered by the MDC PIs Prof. Michael Bader and Prof. Norbert Hübner.

MDC Director Prof. Walter Rosenthal is a member of the executive board of the DZHK. The funding management for the DZHK is handled by a team of five based at the MDC.

Das Deutsche Zentrum für Herz-Kreislauf-Forschung (DZHK) ist eines von sechs Deutschen Zentren der Gesundheitsforschung und wurde 2011 vom Bundesministerium für Bildung und Forschung ins Leben gerufen. Das DZHK ist angetreten, Herz-Kreislauf-Forscher aus der Grundlagen und der klinischen Forschung deutschlandweit zu vernetzen und somit bessere Voraussetzungen für die Translation von Forschungsergebnissen in die klinische Praxis zu schaffen.

Das MDC ist eine von 27 Mitgliedseinrichtungen im DZHK und stellt 9 Principle Investigators (PIs). Es bildet mit der Charité – Universitätsmedizin Berlin, dem Deutschen Herzzentrum Berlin und dem Robert-Koch-Institut den DZHK-Standort „Berlin", einen von sieben Standorten.

Die MDC-PIs sind im Rahmen des DZHK an größeren Standortprojekten im Bereich Gefäßerkrankungen, erbliche und entzündliche Herzerkrankungen, Herzschwäche, Prävention und Bildgebung beteiligt. Darüber forschen sie gemeinsam mit anderen DZHK-Partnern in standortübergreifenden kooperativen Projekten.

MDC-PIs und andere MDC-Wissenschaftler bieten innerhalb des DZHK ihr Knowhow und ihre Expertise als sogenannte „Shared Expertise" an. Mit den Shared Expertise fördert das DZHK Synergien und die Zusammenarbeit der Standorte. Die beiden im DZHK am meisten genutzten Shared Expertise (je 9 mal) „Generierung und kardiovaskuläre Phänotypisierung transgener Ratten" und „Genomics/Proteomics" werden von MDC-PIs Prof. Michael Bader und Prof. Norbert Hübner angeboten.

MDC-Vorstand Prof. Walter Rosenthal ist Vorstandsmitglied des DZHK. Das Fördermittelmanagement für das DZHK wird von einem fünfköpfigen Team mit Sitz am MDC abgewickelt.

EU-Life

Research and innovation sit high on the agenda of the European Union's strategy for the next couple of years. The fragmentation of the research policy landscape, as well as the constant need to promote scientific excellence in Europe, has sparked the creation of a pan-European alliance of research institutes. Thirteen top research institutions (including many of the most prestigious) from thirteen European countries have joined forces and created EU-Life with the aim of promoting scientific excellence.

The goals of this alliance are many and multi-faceted. Primarily it concerns itself with sharing best practices on virtually every topic of interest to the thirteen participating institutes. These exchanges span the fields of scientific strategy, research funding, communication and many other areas of interest. Sharing strategies and policies among the most successful European institutes provides a formidable advantage not only for the MDC but also for the entire European Research area. It will lead to a better understanding of the challenges European life science institutes are currently facing, as well as to better positioning and representing European interests in scientific domains.

The main goal of the alliance is to promote and consolidate the core elements that constitute scientific excellence including cutting-edge technologies, research and infrastructures, internationality, sound administration and ethics, and social responsibility. In order to establish stronger connections and collaborations among researchers in its member institutions, EU-Life is planning its first scientific meeting on the topic of Basic and Translational aspects of Cancer Biology, scheduled to take place in 2014.

All these actions are creating a new powerful bloc that can advocate for the advancement of scientific excellence and integrity in Europe at a variety of levels: from concrete operations at the levels of institutes to the highest realms of policy and strategy at the EU. The MDC is thrilled to contribute its experience and vision in EU-Life and will join the other participating institutes in helping pave the way for a bright future of fundamental research in Europe.

Forschung und Innovation haben einen hohen Stellenwert in der Strategie der Europäischen Union, und das wird auch in den kommenden Jahren so sein. Zugleich ist die forschungspolitische Landschaft Europas fragmentiert. Vor diesem Hintergrund und mit der Aufgabe vor Augen, wissenschaftliche Exzellenz in Europa voranzubringen, haben 13 der renommiertesten Forschungseinrichtungen aus 13 Ländern eine europäische Allianz zur Förderung von Spitzenforschung gegründet: EU-Life.

Die Ziele dieser Allianz sind vielfältig. Ein Hauptaugenmerk liegt auf dem Austausch von „Best Practice"-Beispielen zu buchstäblich allen Fragen, die für die Mitgliedseinrichtungen von Belang sind. Dieser Austausch umfasst die Bereiche Forschungsstrategie, Forschungsförderung und Kommunikation sowie viele weitere Themen. Eine solche Zusammenarbeit erfolgreicher lebenswissenschaftlicher Institute bietet nicht nur dem MDC Vorteile, sondern dient dem gesamten europäischen Forschungsraum. Sie führt zu einem besseren Verständnis der aktuellen Herausforderungen, vor die europäische Life Science-Zentren heute gestellt sind. Zudem sollen europäische Interessen im Bereich der Wissenschaft besser positioniert und präsentiert werden.

Das wesentliche Ziel der Allianz ist, diejenigen Kernelemente zu fördern und zu festigen, die wissenschaftliche Exzellenz ausmachen. Dazu gehören innovative Spitzentechnologien, gute Forschungsinfrastrukturen, Internationalität, eine solide Verwaltung sowie ethische Grundsätze und soziale Verantwortung. Um einen stärkeren Austausch und Kooperationen zwischen den Forschern der verschiedenen Mitgliedseinrichtungen zu etablieren, plant EU-Life sein erstes wissenschaftliches Symposium zu den Grundlagen und translationalen Aspekten der Tumorbiologie. Das Treffen ist für das Jahr 2014 geplant.

All diese Aktivitäten bilden eine neue kraftvolle Bewegung, die für den Fortschritt wissenschaftlicher Exzellenz und Integrität in Europa auf verschiedenen Ebenen eintreten kann: das reicht von konkreten Maßnahmen auf Seiten der Institute bis hin zu den höchsten Ebenen der EU-Politik. Das MDC freut sich sehr, seine Erfahrungen und Vorstellungen in EU-Life miteinbringen zu können und möchte gemeinsam mit den anderen teilnehmenden Instituten den Weg bahnen für eine erfolgreiche Zukunft der Grundlagenforschung in Europa.

Biotechnology Park with Innovation and Founding Center

Biotechnologiepark mit Innovations- und Gründerzentrum

Basic research and clinical research on the campus as well as at neighboring hospitals provide a stimulating environment for the development of the BiotechPark with its Innovation and Founding Center (IGZ).

The BiotechPark Berlin-Buch is one of the largest biotechnology parks in Germany. On about 31,000 square meters, attractive industry-specific laboratories and offices with state-of-the-art equipment are available to company founders and enterprises. Start-ups can grow at the Innovation and Founding Center; additional sites for building on the campus open up options for development. The operating company BBB Management GmbH Campus Berlin-Buch supports the enterprises in all issues and provides a comprehensive service.

Currently, 56 small and medium-sized enterprises conduct research and development at the BiotechPark. Of these, 41 are active in the field of biomedicine. The spectrum of their fields of business comprises medical devices, molecular biology diagnostic products and treatments, pre-clinical pharmacological and pharmacogenomic tests, RNA technologies, search for pharmacologically relevant target molecules, production and assessment of pharmaceuticals as well as analysis and synthesis of biomolecules.

Since 1998, the number of employees working for the biotechnology enterprises has tripled. Currently (status 2014), the companies employ about 775 members of staff. The companies achieve a total annual turnover of 140 million Euro. Their capital stock includes approximately 150 patents, which often derive from cooperations with working groups in the fields of research and clinic applications.

In favor of a sustainable campus development, the BBB has once again actively and intensively promoted the further profiling of the city district Berlin-Buch as a health region and to this end has attracted funding totaling about 500,000 Euro for the years 2011 to 2014. Together with the Buch-based industry stakeholders and in the context of the part-project for site marketing, an umbrella brand has been developed for the entire health location of Buch and numerous communication and marketing measures have been implemented, such as a new web portal, an image brochure, a regularly published site magazine, advertising campaigns and a signposting system for Berlin-Buch.

Grundlagenforschung und klinische Forschung auf dem Campus sowie benachbarte Kliniken bilden ein stimulierendes Umfeld für die Entwicklung des BiotechParks mit seinem Innovations- und Gründerzentrum (IGZ).

Der BiotechPark Berlin-Buch ist einer der größten Biotechparks in Deutschland. Auf rund 31.000 Quadratmetern finden Gründer und Unternehmen günstige branchenspezifische Labor- und Büroflächen mit modernster Ausstattung. Start-ups können im Innovations- und Gründerzentrum wachsen, Baufelder des Campus eröffnen Entwicklungsoptionen. Die Betreibergesellschaft, BBB Management GmbH Campus Berlin-Buch, unterstützt die Unternehmen in allen Belangen und bietet einen umfassenden Service.

Im BiotechPark forschen und entwickeln gegenwärtig 56 kleine und mittelständische Unternehmen. 41 davon sind im Bereich der Biomedizin tätig. Im Spektrum ihrer Geschäftsfelder finden sich medizintechnische Produkte, molekularbiologische Diagnostika und Therapien, präklinische pharmakologische und pharmako-genomische Tests, RNA-Technologien, Suche nach pharmarelevanten Zielmolekülen, Herstellung und Prüfung von Arzneimitteln sowie Analytik und Synthese von Biomolekülen.

Seit 1998 hat sich die Mitarbeiterzahl der Biotech-Unternehmen verdreifacht. Die Firmen beschäftigen derzeit (Stand 2014) etwa 775 Mitarbeiter. Die Unternehmen erzielen jährliche Umsätze von 140 Millionen Euro. Zum Kapitalstock zählen ca. 150 Patente, die oft aus Kooperationen mit Arbeitsgruppen in Forschung und Klinik stammen.

Zugunsten einer nachhaltigen Campusentwicklung hat sich die BBB erneut intensiv in die weitere Profilierung des Stadtteils Berlin-Buch zu einer Gesundheitsregion eingebracht und dafür Fördermittel in Höhe von ca. 500.000 Euro für die Jahre 2011 bis 2014 eingeworben. Gemeinsam mit den Bucher Wirtschaftakteuren wurden im Rahmen des Teilprojekts Standortmarketing eine Dachmarke für den gesamten Gesundheitsstandort Buch entwickelt und zahlreiche Kommunikations- und Werbemaßnahmen realisiert, wie z.B. ein neues Webportal, eine Imagebroschüre, ein regelmäßig erscheinendes Standortmagazin, Anzeigenkampagnen und ein Beschilderungssystem für Berlin-Buch.

The part-project “Development of a utilization concept for select areas on the health location Berlin-Buch as well as recommendations for a marketing strategy and continuation of the master plan for Berlin-Buch” has been successfully completed. As a result, detailed utilization concepts including calculations of profitability and marketing recommendations for numerous areas and buildings in Berlin-Buch have been developed for companies at the BiotechPark that are interested in establishing themselves and that are experiencing strong growth.

At present, the BBB Management GmbH Campus Berlin-Buch employs 34 members of staff. It was established in 1995 as a spin-off company of the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch. Co-partners are the Leibniz-Institut für Molekulare Pharmakologie (FMP) and the Bayer HealthCare Pharmaceuticals, Bayer Pharma AG. Economically independent and a non-profit company with a public mandate, the BBB is a neutral and intermediary partner. It manages the interfaces between research, clinical application, business, administration, politics and the public and is active in competence, support and service networks on both a regional and international level.

The Gläsernes Labor

In 2013, in its capacity as the best-known non-school learning site in Berlin, the Life Science Learning Lab “Gläsernes Labor”, operated by the BBB Management GmbH, offered some 36,500 children, school students, teachers and a broad swathe of the general public demanding laboratory and join-in courses on the topics of genetics, neurobiology, cell biology, ecology, radioactivity and chemistry. Of this number, 12,500 school students attended the laboratory courses on the cam-

Erfolgreich abgeschlossen wurde das Teilprojekt „Entwicklung eines Nutzungskonzepts für ausgewählte Flächen am Gesundheitsstandort Berlin-Buch sowie Empfehlungen für eine Vermarktungsstrategie und ein Fortschreiben des Masterplans für Berlin-Buch“. Im Ergebnis wurden detaillierte Nutzungskonzepte mit Wirtschaftlichkeitsberechnungen und Vermarktungsempfehlungen für zahlreiche Areale und Gebäude in Berlin-Buch für ansiedlungsinteressierte und wachstumsstarke Unternehmen des BiotechParks entwickelt.

Die BBB Management GmbH Campus Berlin-Buch beschäftigt derzeit 34 Mitarbeiter und wurde 1995 aus dem Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch ausgegründet. Mitgesellschafter sind das Leibniz-Institut für Molekulare Pharmakologie (FMP) sowie die Bayer HealthCare Pharmaceuticals, Bayer Pharma AG. Wirtschaftlich selbstständig, nicht Gewinn orientiert und mit öffentlichem Mandat ist die BBB ein neutraler und intermediärer Partner. Sie bedient die Schnittstellen zu Forschung, Klinik, Business, Verwaltung, Politik und Öffentlichkeit und engagiert sich in Kompetenz-, Unterstützungs- und Servicenetzwerken auf regionaler und internationaler Ebene.

Das Gläserne Labor

Das von der BBB Management GmbH betriebene Gläserne Labor hat als bekanntester außerschulischer Lernort Berlins im Jahr 2013 ca. 36.500 Kindern, Schülern, Lehrkräften und einem breiten Laienpublikum anspruchsvolle Labor- und Mitmachkurse zu den Themen Genetik, Neurobiologie, Zellbiologie, Ökologie, Radioaktivität und Chemie angeboten. Davon besuchten 12.500 Schüler die Laborkurse auf dem Campus. Fast 24.000 Kita-Kinder und Schulkinder besuchten Experimentierkurse des



Photo: Guido Rottmann/MDC



Photo: Werner Huthmacher/MDC

pus. Almost 24,000 day care center and school children attended experimentation courses of the "Forschergarten" hosted at Berlin-based day care centers, primary schools as well as at the Gläsernes Labor. The Gläsernes Labor contributed experimentation attractions to numerous events, such as the federal government's open house day, the TSB Schülertage student days and the summer party of the HOWOGE building society, and thereby achieved widespread publicity.

In 2013, the Gläsernes Labor comprehensively expanded its continuing education provision for technicians and scientists. In addition to courses for MDC employees, this was achieved by cooperations with continuing education providers such as C&Q Haberhauffe, Prometheus and the Lette-Verein. In spring 2013, the Gläsernes Labor successfully organized a one-day conference for technicians. In November 2013, an additional fourth laboratory was taken into operation for the continuing education sector, housed in a laboratory building of the BiotechPark.

The MDC has substantially supported these activities, for example, by providing funding for a student laboratory as well as for attractions for the natural science holiday camps for children of employees for improving the compatibility of family and work.

In 2013, on the basis of a long-term agreement with the campus institutions regarding the public relations of the campus, the Gläsernes Labor supervised more than 20 visitor groups of representatives from the worlds of

Forschergartens in Berliner Kitas, Grundschulen sowie im Gläsernen Labor. Das Gläserne Labor beteiligte sich mit Experimentierangeboten an zahlreichen Veranstaltungen, wie dem Tag der offenen Tür der Bundesregierung, den TSB-Schülertagen sowie dem Sommerfest der HOWOGE, und erreichte so eine breite Öffentlichkeit.

2013 hat das Gläserne Labor den Weiterbildungsbereich für technische Angestellte und Wissenschaftler umfangreich ausgebaut. Neben Kursen für MDC-Mitarbeiter wurde dies durch Kooperationen mit Weiterbildern wie C&Q Haberhauffe, Prometheus und dem Lette-Verein erreicht. Im Frühjahr 2013 führte das Gläserne Labor erfolgreich einen eintägigen Kongress für technische Angestellte durch. Im November 2013 wurde für den Weiterbildungsbereich in einem Laborgebäude des BiotechParks zusätzlich ein viertes Labor in Betrieb genommen.

Das MDC hat diese Aktivitäten maßgeblich unterstützt, z.B. durch die Finanzierung eines Schülerlabors sowie von Angeboten zur naturwissenschaftlichen Ferienbetreuung für Mitarbeiterkinder zur Verbesserung der Vereinbarkeit von Beruf und Familie.

Auf Grundlage einer langfristigen Vereinbarung mit den Campus-Einrichtungen zur Öffentlichkeitsarbeit des Campus betreute das Gläserne Labor im Jahre 2013 über 20 Besuchergruppen mit Vertretern aus Wissenschaft, Wirtschaft, Politik und Medien. Dafür organisierte und begleitete es entsprechende Veranstaltungsprogramme. Das Gläserne Labor unterstützte das


Photo: David Ausserhofer/MDC

science, industry, politics and the media. To this end, it organized and supervised corresponding event programs. The Gläsernes Labor also supported the Max Delbrück Center and the Natural Science Museum in organizing and carrying out the "60 Years DNA" conference for teachers. The campus public relations office was responsible for the organization and successful realization of events such as the New Year's Eve reception of the campus institutions and the extremely successful and much attended "Long Night of Sciences". One event highlight was the realization of the 19th Pankower Wirtschaftstag (Pankow Economy Day) in cooperation with the Pankow district authority and including the involvement of partners from the urban district.

In 2012, the employee and image magazine "CampusNews" was further developed to become the site journal "buchinside". In addition to campus-related topics, this publication includes also matters relating to partners in Buch that concern the development of the entire health location. In 2013, three issues were published. Moreover, the BBB actively contributes to the activities and communication platforms of the Berlin-Brandenburg technology cluster and the Berlin Partner network.

Max-Delbrück-Centrum und das Naturkundemuseum auch bei der Organisation und Durchführung des Kongresses „60 Jahre DNA" für Lehrer. Die Campus-Öffentlichkeitsarbeit war verantwortlich für die Organisation und erfolgreiche Durchführung von Veranstaltungen wie dem Neujahrsempfang der Campus-Einrichtungen und der äußerst erfolgreichen und besucherstarken „Langen Nacht der Wissenschaften." Ein Veranstaltungshöhepunkt war die Durchführung des 19. Pankower Wirtschaftstags in Zusammenarbeit mit dem Bezirksamt Pankow und unter Einbeziehung von Partnern aus dem Bezirk.

2012 wurde die Mitarbeiter- und Imagezeitschrift „CampusNews" zum Standortjournal „buchinside" weiterentwickelt. Diese Publikation bezieht neben den Campusthemen auch Themen von Bucher Partnern ein, die die Entwicklung des gesamten Gesundheitsstandorts betreffen. 2013 erschienen drei Ausgaben. Darüber hinaus bringt sich die BBB aktiv in die Aktivitäten und Kommunikationsplattformen des Berlin-Brandenburger Technologie-Clusters sowie des Netzwerkes von Berlin Partner ein.

Health Care Industry Cluster

Cluster Gesundheitswirtschaft

The life sciences and health care industry play a leading role in the Berlin-Brandenburg region. More than 300,000 people work in the wider field of health care; the industry sector achieves an annual turnover of about 16 billion Euro. The respective federal state governments of Berlin and Brandenburg have identified the significance of the "health care region" very early on and approved a master plan in October 2007. This plan was evaluated and reviewed in 2012. The management board of the "Health Care Industry Cluster" is responsible for implementing this master plan. The aim of the cluster and its stakeholders is to develop the Berlin-Brandenburg region to become the leading site for managed health care in Germany. In late 2013, Walter Rosenthal, Scientific Director and Chair of the Board of Directors of the MDC, was appointed spokesman for the cluster.

For the MDC in its capacity as a center for basic research, the fast transfer of knowledge from the world of science to clinical and industrial applications is of great importance. This comprises far more than merely the development of new active agents and the establishment of spin-off enterprises. This is about new treatment strategies, different tools for diagnosis and prevention of diseases right up to medical rehabilitation. The MDC and, since 2013, the Berlin Institute of Health (BIH – a cooperation of the Charité – Universitätsmedizin Berlin with the MDC) are both interested in such a transfer in the sense of providing "translational medicine".

Some figures for the Berlin-Brandenburg health care region:
- 19 Berlin-based and 6 Brandenburg-based universities with some 170 health-related study courses;
- more than a dozen extramural research institutions focusing on the life sciences;
- approximately 30 pharmaceutical companies with a total of about 10,000 employees;
- about 280 medical engineering companies with a total of more than 11,600 employees;
- about 220 biotechnology companies with a total of about 4,300 employees;
- approximately 5,900 health care industry enterprises;
- 132 hospitals with a total of more than 35,000 beds;
- the largest university hospital in Europe: the Charité – Universitätsmedizin Berlin;
- more than 3,000 clinical studies per year.

More information: www.healthcapital.de
(available only in German language)

Die Lebenswissenschaften und die Gesundheitswirtschaft spielen in der Region Berlin-Brandenburg eine führende Rolle. Mehr als 300.000 Menschen arbeiten im Bereich der Gesundheitswirtschaft; die Branche macht einen Jahresumsatz von rund 16 Milliarden Euro. Die Landesregierungen von Berlin und Brandenburg haben die Bedeutung der „Gesundheitsregion" sehr früh erkannt und im Oktober 2007 einen Masterplan verabschiedet. Er wurde 2012 evaluiert und überarbeitet. Mit der Umsetzung des Masterplanes ist das Management des „Clusters Gesundheitswirtschaft" betraut. Ziel des Clusters und seiner Akteure ist es, die Region Berlin-Brandenburg zum führenden deutschen Gesundheitsstandort zu entwickeln. Sprecher des Clusters ist seit Ende 2013 der wissenschaftliche Direktor und Stiftungsvorstand des MDC, Walter Rosenthal.

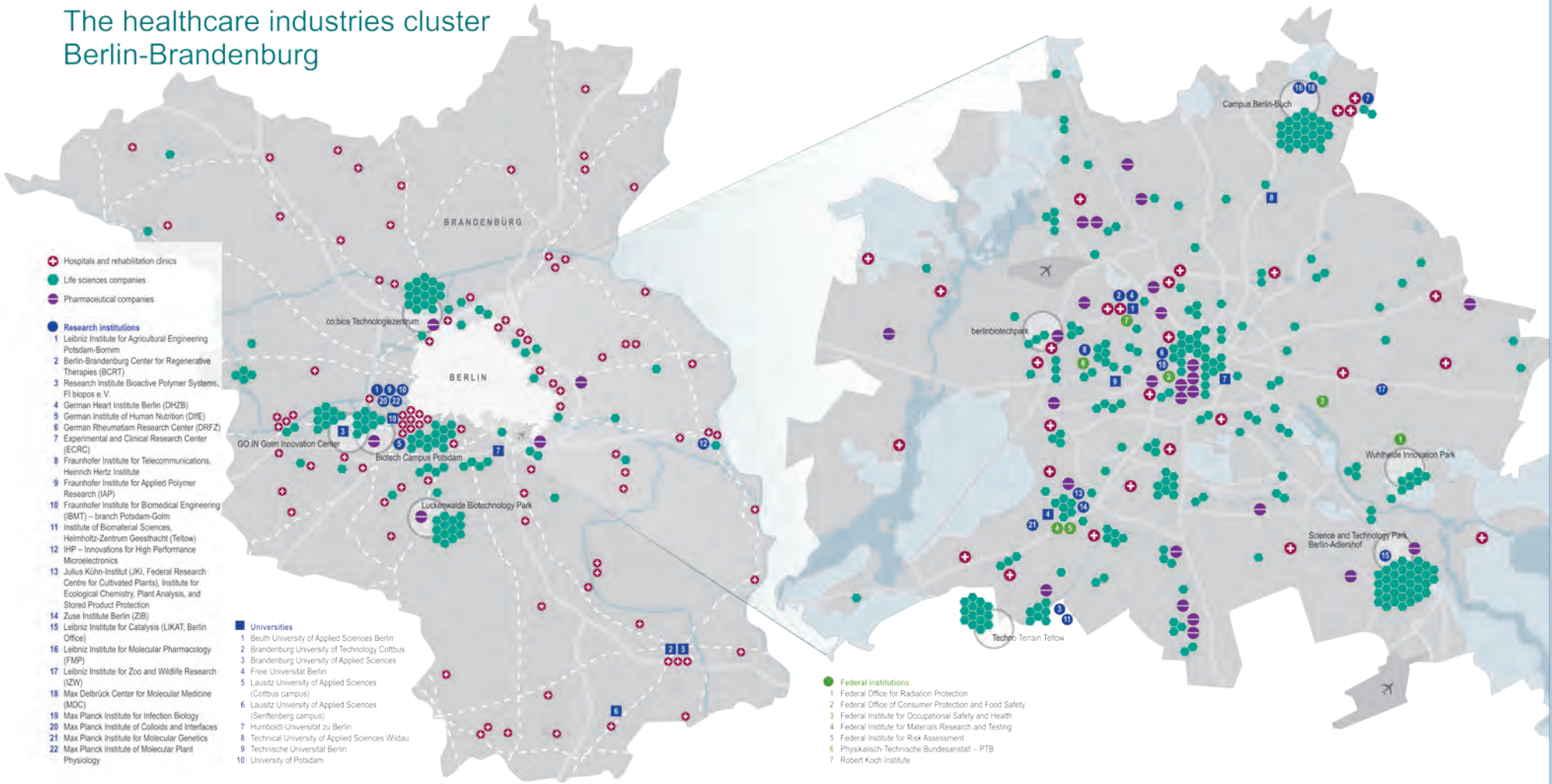
Für das MDC als Zentrum der Grundlagenforschung ist ein schneller Transfer von der Wissenschaft in die Klinik und die Wirtschaft wichtig. Dabei geht es um weit mehr als nur die Entwicklung neuer Wirkstoffe oder die Gründung von Spin-offs. Es geht um neue Behandlungsstrategien, andere Diagnose-Werkzeuge und Prävention von Krankheiten bis hin zur Rehabilitation. Das MDC und seit 2013 das Berliner Institut für Gesundheitsforschung/Berlin Institute of Health sind an einem solchen Transfer im Sinne „translationaler Medizin" interessiert.

Einige Zahlen zur Gesundheitsregion Berlin-Brandenburg
- 19 Berliner und 6 Brandenburger Hochschulen mit rund 170 gesundheitsbezogenen Studiengängen
- mehr als ein Dutzend außeruniversitäre Forschungseinrichtungen mit Schwerpunkt in den Lebenswissenschaften
- ca. 30 Pharmaunternehmen mit rund 10.000 Beschäftigten
- etwa 280 Medizintechnikunternehmen mit mehr als 11.600 Beschäftigten
- etwa 220 Biotech-Unternehmen mit ca. 4.300 Beschäftigten
- ca. 5.900 Unternehmen der Gesundheitswirtschaft
- 132 Kliniken mit mehr als 35.000 Betten
- größte Uniklinik Europas: die Charité – Universitätsmedizin Berlin
- mehr als 3.000 klinische Studien pro Jahr

Mehr: www.healthcapital.de

Source / Quelle: „Life Sciences Brochure", Berlin Partner für Wirtschaft und Technologie

The healthcare industries cluster Berlin-Brandenburg



Press Department

Pressestelle

During 2012/2013 the MDC Press Department published 135 press releases in German and English and informed the media about research results, developments and events. These resulted in about 90 interview requests. MDC news were covered by newspapers, weeklies, magazines, radio and television in hundreds of articles and reports both in Germany and abroad.

Among them were the BBC World Service, La Chaîne Info (French TV group), La Pagina (El Salvador). El Correo (Bilbao, Spain), El Heraldo, El Mexicano (Mexico), Le Monde (Paris, France), German public service broadcasters such as ARD, 3Sat, Hessischer Rundfunk (HR), Mitteldeutscher Rundfunk (MDR), Rundfunk-Berlin-Brandenburg (rbb), Deutschlandfunk (DLF), Deutschlandradio Wissen, Deutschlandradio Kultur, Westdeutscher Rundfunk (WDR5), private broadcasters such as N24, RTL and magazines like Focus, Spiegel, Stern (here all online), and newspapers such as the Frankfurter Allgemeine Zeitung (FAZ), Der Standard (Vienna, Austria), Süddeutsche Zeitung, Die Welt, the German newswire service DPA as well as Die Zeit, Laborjournal, Science and Nature. In the above-mentioned period the Press Department published seven press reviews documenting part of this coverage.

Three topics of particular importance in 2012 were the Leibniz Award for Professor Nikolaus Rajewsky, the founding of the German Center for Cardiovascular Research (DZHK) and the plans of the Federal Ministry of Education and Research (BMBF) at that time on the future cooperation of MDC and Charité – now Berlin Institute of Health (BIH), see below). Especially for the latter two topics, the MDC Press Department organized many interviews with the MDC director as well as with the designated BIH Chairman. Also notable was the 20th anniversary of the MDC.

Broad media coverage was given to the MDC press work about scientific publications of MDC researchers. Among these was the research of Professor Gary R. Lewin, who showed that a gene mutation leads to the impairment of two senses – touch and hearing, findings published in *PLOS Biology.* Also of great interest to the media was the research of Marion Bimmler, who showed in a study published in *PLOS ONE* that autoantibodies can damage blood vessels in the brain, an important factor in the development of Alzheimer's dis-

Die MDC-Pressestelle informierte 2012/2013 in 135 Pressemitteilungen (Deutsch/Englisch) die Medien über Forschungsergebnisse, Entwicklungen und Ereignisse. Sie waren Anlass für rund 90 Interviewanfragen. Die Informationen der Pressestelle fanden Widerhall in hunderten von Beiträgen von Zeitungen, Wochenzeitungen, Magazinen, Funk- und Fernsehen im In- und Ausland.

Unter ihnen waren BBC World Service, La Chaîne Info (französische TV-Gruppe), La Pagina (El Salvador). El Correo (Bilbao, Spanien), El Heraldo, El Mexicano (Mexiko), Le Monde (Paris, Frankreich), öffentlich-rechtliche Rundfunkanstalten wie ARD, 3Sat, Hessischer Rundfunk (HR), Mitteldeutscher Rundfunk (MDR), Rundfunk-Berlin-Brandenburg (rbb), Deutschlandfunk (DLF), Deutschlandradio Wissen, Deutschlandradio Kultur, Westdeutscher Rundfunk (WDR5), Privatsender wie N24 und RTL, Magazine wie Focus, Spiegel, Stern (alle online), Tageszeitungen darunter Frankfurter Allgemeine Zeitung (FAZ), Der Standard (Wien, Österreich), Süddeutsche Zeitung, Die Welt, sowie Deutsche Presse-Agentur (dpa), Die Zeit, Laborjournal und die Wissenschaftsmagazine Science und Nature. Im genannten Zeitraum gab die Pressestelle sieben Pressespiegel heraus, in denen ein Teil der Medienberichterstattung dokumentiert ist.

Von besonderer Bedeutung 2012 waren der Leibniz-Preis für Prof. Nikolaus Rajewsky, die Eröffnung des Deutschen Zentrums für Herz-Kreislaufforschung (DZHK) und die Pläne des Bundesforschungsministeriums (BMBF) über die künftige Kooperation von MDC und Charité – jetzt Berliner Institut für Gesundheitsforschung (BIG) (s.u.). Besonders zu den beiden zuletzt genannten Themen vermittelte die MDC-Pressestelle zahlreiche Interviews mit dem MDC-Vorstand sowie dem designierten Vorstand des Berliner Instituts für Gesundheitsforschung. Beachtet wurde auch das 20-jährige Bestehen des MDC.

Auf ein breites Medienecho stieß 2012 die MDC-Pressearbeit über wissenschaftliche Publikationen von MDC-Forscherinnen und -Forschern. Darunter war eine in *PLOS Biology* veröffentlichte Arbeit von Prof. Gary R. Lewin, der zeigte, dass eine Genmutation zum Verlust sowohl des Gehör- als auch des Tastsinns führt. Großes Presseecho fand die Forschungsarbeit von Marion Bimmler, die in einer in *PLOS ONE* veröffentlichten

Opening ceremony of the Berlin Institute of Health (BIH) on June 18, 2013 in Berlin-Mitte (from left)
Eröffnungsfeier für das Berliner Institut für Gesundheitsforschung (BIG) am 18. Juni 2013 in Berlin-Mitte (v. l.):

Prof. Karl Max Einhäupl (CEO of the Charité/Vorstandsvorsitzender der Charité), Dr. Georg Schütte (State Secretary of the Federal Ministry of Education and Research/Staatssekretär im Bundesforschungsministerium), Prof. Ernst Rietschel (Chairman, BIH/BIG-Vorstandsvorsitzender), Prof. Johanna Wanka (Federal Minister of Education and Research/Bundesforschungsministerin), Prof. Walter Rosenthal (Chairman of the Board and Scientific Director of the MDC/Vorstandsvorsitzender und wissenschaftlicher Stiftungsvorstand des MDC) and/und Prof. Annette Grüters-Kieslich (Dean of the Charité Medical Faculty/Dekanin der Medizinischen Fakultät der Charité).



Photo: David Ausserhofer/BIG

ease and dementia. The findings of Professor Helmut Kettenmann and colleagues of the Charité, published in *Nature Medicine,* also received much media coverage. They showed that neural precursor cells induce cell death in certain brain tumors. Also well covered by the media was a new approach to treat life-threatening acute liver failure, developed by Dr. Stefan Donath, a physician and researcher, published in *Hepatology.*

The outstanding event in 2013 was the opening of the BIH, which received high media attention. In coordination with the (then designated) Chairman of the BIH, the Office of the Scientific Director of the MDC and the Corporate Communications Division of the Charité, the MDC Press Department carried out the entire press work for the opening of the BIH, wrote the invitations for the media, the press release and the background information, and arranged numerous interviews.

Well covered by the media in 2013 was the MDC press work on the findings, published in *Nature,* of Professor Dominik Müller and colleagues from Yale, Harvard, and MIT in the USA, who showed that increased dietary salt intake may drive the development of autoimmune diseases. Broad media coverage, especially in France, was also given to Dr. Michael Sieweke's (Inserm and MDC) *Nature* publication about the ability of blood stem cells to respond to emergencies, and to Dr. James Poulet, who received the Paul Ehrlich and Ludwig Darmstaedter Prize for Young Researchers in St. Paul's Church in Frankfurt am Main.

Furthermore, the MDC Press Department was responsible for the press work for the opening of the new laboratory building of the MDC, the Max Rubner House, with an accompanying photo exhibition there. Together with the Press Department of the German Cancer Research Center (DKFZ), the MDC Press Department invited media representatives to a press conference in Berlin on the occasion of the founding of the German Stem Cell Network (GSCN) and moderated this conference together with a DKFZ colleague.

Press Department staff:
Barbara Bachtler (head) and
Ann-Kathrin Schöpflin (team assistant)

Studie nachwies, dass Autoantikörper Blutgefäße im Gehirn schädigen können, ein wichtiger Faktor für die Entstehung von Alzheimer und Demenz. Von großem Interesse für die Medien war eine von Prof. Helmut Kettenmann und Kollegen von der Charité in *Nature Medicine* publizierte Arbeit, in der er zeigte, wie neuronale Stammzellen des Gehirns Hirntumoren zerstören. Auch die Forschung von Dr. Stefan Donath über einen Therapieansatz gegen das lebensbedrohliche akute Leberversagen, publiziert in *Hepatology,* fand Beachtung.

Herausragendes Ereignis 2013 war die Eröffnung des Berliner Instituts für Gesundheitsforschung/Berlin Institute of Health (BIG/BIH), über das die Medien groß berichteten. In Abstimmung mit dem (damals designierten) Vorstandsvorsitzenden des BIG, dem Büro des wissenschaftlichen Vorstands des MDC und der Charité-Unternehmenskommunikation koordinierte die MDC-Pressestelle die gesamte Pressearbeit für die Eröffnung, verfasste Presseeinladung, Pressemitteilung sowie Hintergrundinformationen und vermittelte zahlreiche Interviews.

Auf große Medienresonanz fiel 2013 auch die MDC-Pressearbeit über die Forschung von Prof. Dominik Müller und Kollegen von Yale, Harvard und MIT in den USA, die in einer in *Nature*-Publikation zeigten, dass zu viel Kochsalz die Entstehung von Autoimmunerkrankungen fördern könnte. Auf ein breites Presseecho, vor allem in Frankreich, stieß die *Nature*-Publikation von Dr. Michael Sieweke (INSERM, MDC), der einen körpereigenen Notfallmechanismus für die Blutbildung bei Infektionen entdeckte. Breit berichtet wurde auch über Dr. James Poulet, der in der Frankfurter Paulskirche den Paul Ehrlich- and Ludwig Darmstaedter-Preis für Nachwuchswissenschaftler erhielt.

Weiter machte die MDC-Pressestelle die Pressearbeit zur Eröffnung des Max-Rubner-Hauses des MDC mit einer begleitenden Fotoausstellung. Anlässlich der Gründung des deutschen Stammzellnetzwerks lud die MDC-Pressestelle gemeinsam mit der Pressestelle des Deutschen Krebsforschungszentrums (DKFZ) die Medien zu einer Pressekonferenz in Berlin ein, die sie gemeinsam mit einer DKFZ-Kollegin moderierte.

Mitarbeiter der Pressestelle:
Barbara Bachtler (Leitung),
Ann-Kathrin Schöpflin (Teamassistenz)

Communications Department

Kommunikationsabteilung

In 2012 and 2013, the communications department of the MDC organized about two dozen events. These included 13 scientific conferences, the opening of the Berlin Institute of Health (in cooperation with the event management department of the Charité) and the ceremony for celebrating the 20-year anniversary of the Max Delbrück Center with more than 600 guests. The guests invited to the anniversary celebrations included the then Federal Minister of Education and Research, Annette Schavan, the Berlin Science Senator, Cornelia Yzer, as well as many members of the German Bundestag and House of Representatives. In the years under review, the department also supervised visits from high-ranking politicians and guests of state, including the President of the Republic of Singapore,Dr. Tony Tan, and the Chinese Ambassador to Germany, Shi Mingde, and it organized a parliamentary evening as well as an open house day. An aspect of the department's work that holds extraordinary public appeal is the organization of the annual Long Night of Sciences comprising guided tours of the laboratories, science slams and other events. Each year, these events are attended by several thousands of people visiting the campus. The communications department organizes also the CareerDay, which takes place once a year.

In early 2012, the MDC became the target of a campaign launched by protesters against animal experiments. The trigger for this were the plans for a replacement building for two old animal houses, where the keeping of animals and laboratories are to be combined according to the latest standards (IPL – In vivo Pathophysiology Laboratory). In consequence, the MDC further increased its already proactive communication regarding the work with laboratory animals. The communications department provides comprehensive online information on the topic of animal experimentation, it organizes guided tours on the topic during the Long Nights of Science and it coordinates the communication with the media and politics regarding the topic of animal experimentation. To this end, for instance, numerous meetings with federal state and federal government politicians have been organized as well as a parliamentary evening and laboratory visits for media and political representatives. The topic is also actively addressed at schools, where staff members of the communications department and scientists enter into discussion with the students.

Die Kommunikationsabteilung des MDC organisierte in den Jahren 2012 und 2013 rund zwei Dutzend Veranstaltungen. Dazu zählten 13 wissenschaftliche Konferenzen, die Eröffnung des Berliner Instituts für Gesundheitsforschung/Berlin Institute of Health (in Kooperation mit dem Veranstaltungsmanagement der Charité) und der Festakt zum 20-jährigen Bestehen des Max-Delbrück-Centrums mit mehr als 600 Gästen. Unter den Gästen des Festaktes waren die damalige Bundesforschungsministerin Annette Schavan, die Berliner Forschungssenatorin Cornelia Yzer sowie viele Abgeordnete des Bundestags und des Abgeordnetenhauses. Hinzu kamen in den Berichtsjahren von der Abteilung betreute Besuche von hochrangigen Politikern und Staatsgästen, darunter der Staatspräsident von Singapur, Dr. Tony Tan, und der chinesische Botschafter in Deutschland, Shi Mingde, sowie ein Parlamentarischer Abend und ein Tag der offenen Tür. Ein außerordentlich publikumswirksamer Aspekt der Arbeit ist die Organisation der jährlich stattfindenden Langen Nacht der Wissenschaften mit Laborführungen, Science Slams und weiteren Aktionen. Zu diesen Veranstaltungen kommen jeweils mehrere Tausend Menschen auf den Campus. Auch der CareerDay, der jährlich stattfindet, wird von der Kommunikationsabteilung organisiert.

Das MDC wurde Anfang 2012 Ziel einer Kampagne von Tierversuchsgegnern. Auslöser waren die Pläne für einen Ersatzbau von zwei alten Tierhäusern, in dem Haltung und Labore nach modernsten Standards kombiniert werden (In-vivo-Pathophysiologie-Labor, IPL). Daraufhin verstärkte das MDC seine bis dahin bereits pro-aktive Kommunikation zu der Arbeit mit Versuchstieren. Die Kommunikationsabteilung stellt umfangreiche Informationen zum Thema Tierversuche online zur Verfügung, sie organisiert Führungen dazu bei den Langen Nächten der Wissenschaften und koordiniert die mediale und politische Kommunikation zum Thema Tierversuche. So gab es dazu zahlreiche Treffen mit Landes- und Bundespolitikern, einen Parlamentarischen Abend und Laborbesuche durch Medien und Politik. Das Thema wird auch aktiv in Schulen getragen, wo Mitglieder der Kommunikationsabteilung und Wissenschaftler mit Schülerinnen und Schülern diskutieren.

Zu den Aufgaben der Abteilung gehören Print- und Onlinepublikationen (Corporate Publishing) aller



Photo: Guido Rottmann/MDC

Networking at the MDC Careerday.

The department's tasks include print and online publications (corporate publishing) of all kinds, including also the research report at hand. During the period under review, a total of 20 publications were published, ranging from the employee magazine imdc, over leaflets and thematic brochures, to a photo brochure and a 350-page popular science book about a scientific investigation of the genetic fundamentals of high blood pressure that went on for more than 20 years ("The Case of the Short-fingered Musketeer"). The communications department coordinated also a 44-page special issue of the "bild der wissenschaft" magazine on occasion of the MDC's 20-year anniversary. In addition, it supervised the conference proceedings of several scientific conferences.

In the context of a strategic realignment of the communication strategy, the employee magazine imdc was discontinued by the middle of 2013 so as to instead place more emphasis on the online presence. Since May 2013, the MDC's intranet includes a news center and, since spring 2014, the online service includes MDC Insights, the MDC's news center for external visitors to our website. We offer also the service of an electronic newsletter. The MDC maintains a presence on the most important social media platforms Facebook, Twitter, LinkedIn and Xing and is particularly active on Facebook and Twitter.

In its capacity as a publicly funded institution for basic research, the MDC is aware of its obligation to contribute to an improved understanding of science in the public, in particular amongst school students. To this end, the MDC offers a continuing education program for teachers that has received much notice throughout Germany: Labor trifft Lehrer (LTL – Laboratory Meets Teacher). On occasion of the 60th anniversary of the publication of the structure of DNA, the LTL and cooperation partners organized 13 continuing education courses for teachers, 7 school student workshops and a large-scale conference.

To strengthen the community spirit at the MDC (community building), the communications department is involved in the weekly social evenings (beer hour), organizes a glühwein stall during the Christmas season and cooperates in the project group Career and Family. The GreenCampus campaign for the economical use of resources likewise derives from an initiative of the communications department

Art, darunter auch der vorliegende Research Report. Im Berichtszeitraum erschienen insgesamt 20 Publikationen, vom Mitarbeitermagazin imdc über Flyer und Themenbroschüren (Ausbildung, Beruf & Familie) bis hin zu einer Fotobroschüre und einem populärwissenschaftlen 350-Seiten-Buch über eine mehr als 20 Jahre währende Studie zu genetischen Grundlagen von Bluthochdruck („The Case of the Short-fingered Musketeer"). Die Abteilung Kommunikation koordinierte auch eine 44-seitige Sonderveröffentlichung von „bild der wissenschaft" anlässlich des 20-jährigen Bestehens des MDC. Auch mehrere Tagungsbände zu wissenschaftlichen Konferenzen wurden betreut.

Im Zuge einer strategischen Neuausrichtung der Kommunikation wurde Mitte 2013 das Mitarbeitermagazin imdc eingestellt und dafür eine verstärkte Online-Präsenz geschaffen. Das Intranet des MDC verfügt seit Mai 2013 über ein Nachrichtenzentrum, und seit Frühjahr 2014 gibt es MDC Insights, das Nachrichtenzentrum des MDC für externe Besucher unserer Webseite. Auch einen elektronischen Newsletter bieten wir an. Das MDC unterhält Präsenzen auf den wichtigsten Social Media-Plattformen Facebook, Twitter, LinkedIn und Xing und engagiert sich insbesondere auf Facebook und Twitter.

Als öffentlich geförderte Einrichtung der Grundlagenforschung sieht sich das MDC in der Pflicht, zu einem besseren Verständnis von Wissenschaft in der Öffentlichkeit, insbesondere bei Schülerinnen und Schülern, beizutragen. Hierzu bietet das MDC ein bundesweit viel beachtetes Lehrerfortbildungsprogramm an: Labor trifft Lehrer (LTL). 13 Lehrerfortbildungen, 7 Schülerworkshops und eine große Konferenz anlässlich des 60. Jahrestages der Veröffentlichung der DNA-Struktur wurden von LTL und Kooperationspartnern organisiert.

Um das Gemeinschaftsgefühl am MDC zu stärken (Community Building), engagiert sich die Kommunikationsabteilung bei den wöchentlichen sozialen Abenden (Beer Hour), mit einem Glühweinstand zur Adventszeit und durch die Mitarbeit in der Projektgruppe Beruf und Familie. Auch die GreenCampus-Kampagne zum sparsamen Umgang mit Ressourcen geht auf die Initiative der Kommunikationsabteilung zurück.

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Issue: April, 2014
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Organizational Structure

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The Max-Delbrück Center for Molecular Medicine (MDC) Berlin-Buch is a foundation under public law of the State of Berlin with the purpose of pursuing medical research at the molecular and cellular levels and implementing its clinical and practical application.

Das Max-Delbrück Centrum für Molekulare Medizin (MDC) Berlin-Buch ist eine Stiftung des öffentlichen Rechts des Landes Berlin. Ihr Zweck ist es, medizinische Forschung auf molekularer und zellulärer Ebene zu betreiben und ihre klinische Anwendung und praktische Umsetzung zu voranzubringen.

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The Board of Trustees is the supervisory body of the MDC and monitors the conduct of operations with respect to legality, appropriateness, economic efficiency and financial viability. The Board decides on general research objectives as well as important research policy and financial matters of the Foundation

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The Scientific Committee of the Board of Trustees prepares the decisions of the Board of Trustees in scientific matters. The Scientific Committee is responsible for the ongoing evaluation of the results of the research work of the MDC through scientific assessment. Together with the scientific members of the Board of Trustees, up to seven external specialists sit on the Scientific Committee.

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Der Wissenschaftliche Ausschuss des Kuratoriums bereitet die Entscheidungen des Kuratoriums in wissenschaftlichen Fragen vor. Er trägt die Verantwortung für die fortlaufende Ergebnisbewertung der Forschungsarbeiten des MDC durch wissenschaftliche Begutachtung. Dem Wissenschaftlichen Ausschuss gehören neben den wissenschaftlichen Mitgliedern des Kuratoriums bis zu sieben externe Fachwissenschaftler an.

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Der Stiftungsvorstand leitet das Institut und besteht aus einem wissenschaftlichen Mitglied, Prof. Dr. Walter Rosenthal, und einem administrativen Mitglied, Dr. Heike Wolke. Vorsitzender des Stiftungsvorstandes ist Prof. Dr. Walter Rosenthal.

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The Staff Council is the collective representation of the employees of the MDC, based on the Staff Representation Act of Berlin.

The Staff Council is involved in decisions regarding personnel and social matters and monitors the observance of labor rights, which are regulated in laws, collective agreements, internal agreements and administrative provisions. The Staff Council receives suggestions and complaints of the employees and clarifies these together with the responsible departments or the Executive Board.

The Staff Council currently has 13 members from all employee groups at the MDC.

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Der Personalrat ist auf der Grundlage des Personalvertretungsgesetzes des Landes Berlin die kollektive Interessenvertretung der Mitarbeiterinnen und Mitarbeiter des MDC.

Der Personalrat ist an Entscheidungen zu personellen und sozialen Belangen beteiligt und überwacht die Einhaltung von Arbeitnehmerrechten, die in Gesetzen, Tarifverträgen, Dienstvereinbarungen und Verwaltungsvorschriften geregelt sind. Er nimmt Anregungen und Beschwerden der Mitarbeiter entgegen und klärt diese in Zusammenarbeit mit den zuständigen Abteilungen oder dem Vorstand.

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Publications

MDC Publications 2012/2013

	Total number of publications (articles and reviews)	High Impact Papers (IF>=10)
2013	404	57*
2012	379	48

Source: MDC Repository; As of 31.01.2014 * IF as of 2012

Ten most cited MDC publications 2012

Rank	ID (Web of Science)	Document type	Reference
1	WOS:000300261700009	Review	Gherardi, E.*, **Birchmeier, W.*, Birchmeier, C.,** and Woude, G.V. (2012). Targeting MET in cancer: rationale and progress. Nat Rev Cancer 12, 89-103. *Joint first authors
2	WOS:000305095400016	Article	**Baltz, A.G.*, Munschauer, M.*, Schwanhaeusser, B., Vasile, A., Murakawa, Y., Schueler, M.,** Youngs, N., Penfold-Brown, D., Drew, K., **Milek, M., Wyler, E.,** Bonneau, R., **Selbach, M., Dieterich, C.,** and **Landthaler, M.** (2012). The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. Mol Cell 46, 674-690. *Joint first authors
3	WOS:000298733500013	Article	**Friedlaender, M.R.*, Mackowiak, S.D.*, Li, N., Chen, W.,** and **Rajewsky, N.** (2012). miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. Nucleic Acids Res 40, 37-52. *Joint first authors
4	WOS:000299115700008	Article	Nickolas, T.L., **Schmidt-Ott, K.M.,** Canetta, P., Forster, C., Singer, E., Sise, M., Elger, A., Maarouf, O., Sola-Del Valle, D.A., O'Rourke, M., Sherman, E., Lee, P., Geara, A., Imus, P., Guddati, A., Polland, A., Rahman, W., Elitok, S., Malik, N., Giglio, J., El-Sayegh, S., Devarajan, P., Hebbar, S., Saggi, S.J., Hahn, B., Kettritz, R., Luft, F.C., and Barasch, J. (2012). Diagnostic and prognostic stratification in the emergency department using urinary biomarkers of nephron damage a multicenter prospective cohort study. J Am Coll Cardiol 59, 246-255.
5	WOS:000298414100017	Article	**Bieschke, J.*, Herbst, M.*, Wiglenda, T., Friedrich, R.P., Boeddrich, A., Schiele, F., Kleckers, D.,** Lopez Del Amo, J.M., Gruening, B.A., Wang, Q., **Schmidt, M.R.,** Lurz, R., Anwyl, R., **Schnoegl, S.,** Faendrich, M., Frank, R.F., Reif, B., Guenther, S., Walsh, D.M., and **Wanker, E.E.** (2012). Small-molecule conversion of toxic oligomers to nontoxic βsheet-rich amyloid fibrils. Nat Chem Biol 8, 93-101. *Joint first authors
6	WOS:000300793100009	Article	**Poulet, J.F.A.*,** Fernandez, L.M.J.*, Crochet, S.*, and Petersen, C.C.H. (2012). Thalamic control of cortical states. Nat Neurosci 15, 370-372. *Joint first authors
7	WOS:000298601300027	Article	**Anders, G., Mackowiak, S.D., Jens, M., Maaskola, J., Kuntzagk, A., Rajewsky, N., Landthaler, M.,** and **Dieterich, C.** (2012). doRiNA: a database of RNA interactions in post-transcriptional regulation. Nucleic Acids Res 40, D180-D186.
8	WOS:000307686000006	Article	**Sander, S., Calado, D.P.,** Srinivasan, L., **Koechert, K.,** Zhang, B., Rosolowski, M., Rodig, S.J., Holzmann, K., Stilgenbauer, S., Siebert, R., Bullinger, L., and **Rajewsky, K.** (2012). Synergy between PI3K signaling and MYC in Burkitt lymphomagenesis. Cancer Cell 22, 167-179.
9	WOS:000303164400031	Article	**Zhelyazkova, P.,** Hammani, K., Rojas, M., Voelker, R., Vargas-Suarez, M., Boerner, T., and Barkan, A. (2012). Protein-mediated protection as the predominant mechanism for defining processed mRNA termini in land plant chloroplasts. Nucleic Acids Res 40, 3092-3105.
10	WOS:000303763500044	Article	Guo, W.*, **Schafer, S.*,** Greaser, M.L., **Radke, M.H., Liss, M., Govindarajan, T., Maatz, H., Schulz, H.,** Lincoln, S.E., Parrish, A.M., **Dauksaite, V., Vakeel, P., Klaassen, S., Gerull, B., Thierfelder, L.,** Regitz-Zagrosek, V., Hacker, T.A., Saupe, K.W., Dec, G.W., Ellinor, P.T., MacRae, C.A., Spallek, B., Fischer, R., Perrot, A., Ozcelik, C., **Saar, K., Hubner, N.,** and **Gotthardt, M.** (2012). RBM20, a gene for hereditary cardiomyopathy, regulates titin splicing. Nat Med 18, 766-773. *Joint first authors

Source: ISI Web of Science; As of 14.04.2014

Ten most cited MDC publications 2013

Rank	ID (Web of Science)	Document type	Reference
1	WOS:000316650500035	Article	**Memczak, S.*, Jens, M.*, Elefsinioti, A.*, Torti, F.*, Krueger, J., Rybak, A., Maier, L., Mackowiak, S.D., Gregersen, L.H., Munschauer, M., Loewer, A., Ziebold, U., Landthaler, M., Kocks, C., le Noble, F.,** and **Rajewsky, N.** (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495, 333-338. *Joint first authors
2	WOS:000313404600004	Review	**Kettenmann, H.,** Kirchhoff, F., and Verkhratsky, A. (2013). Microglia: new roles for the synaptic stripper. Neuron 77, 10-18.
3	WOS:000315733400001	Review	Santos, R.A.S., Ferreira, A.J., Verano-Braga, T., and **Bader, M.** (2013). Angiotensin-converting enzyme 2, angiotensin-(1-7) and Mas: new players of the renin angiotensin system. J Endocrinol 216, R1-R17.
4	WOS:000317886100016	Article	**Holland, J.D., Klaus, A.,** Garratt, A.N., and **Birchmeier, W.** (2013). Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol 25, 254-264.
5	WOS:000314263600035	Article	Floegel, A., Stefan, N., Yu, Z., Muehlenbruch, K., Drogan, D., Joost, H.G., Fritsche, A., Haering, H.U., Hrabe de Angelis, M., Peters, A., Roden, M., Prehn, C., Wang-Sattler, R., Illig, T., Schulze, M.B., Adamski, J., Boeing, H., and **Pischon, T.** (2013). Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes 62, 639-648.
6	WOS:000313046500034	Article	**Carlo, A.S.,** Gustafsen, C., **Mastrobuoni, G.,** Nielsen, M.S., **Burgert, T.,** Hartl, D., **Rohe, M.,** Nykjaer, A., Herz, J., Heeren, J., **Kempa, S.,** Petersen, C.M., and **Willnow, T.E.** (2013). The pro-neurotrophin receptor sortilin is a major neuronal apolipoprotein E receptor for catabolism of amyloid-β peptide in the brain. J Neurosci 33, 358-370.
7	WOS:000313793700010	Article	Scholz, C.W., Pinto, A., Linkesch, W., Linden, O., Viardot, A., Keller, U., Hess, G., Lastoria, S., Lerch, K., Frigeri, F., Arcamone, M., Stroux, A., Frericks, B., Pott, C., and **Pezzutto, A.** (2013). ^{90}yttrium-ibritumomab-tiuxetan as first-line treatment for follicular lymphoma: 30 months of follow-up data from an international multicenter phase II clinical trial. J Clin Oncol 31, 308-313.
8	WOS:000318558200036	Article	Mossadegh-Keller, N., Sarrazin, S., Kandalla, P.K., Espinosa, L., Stanley, E.R., Nutt, S.L., Moore, J., and **Sieweke, M.H.** (2013). M-CSF instructs myeloid lineage fate in single haematopoietic stem cells. Nature 497, 239-243.
9	WOS:000317898000084	Article	**Krabbe, G.*,** Halle, A.*, **Matyash, V.,** Rinnenthal, J.L., Eom, G.D., Bernhardt, U., Miller, K.R., Prokop, S., **Kettenmann, H.#,** and Heppner, F.L.# (2013). Functional impairment of microglia coincides with beta-amyloid deposition in mice with Alzheimer-like pathology. PLoS ONE 8, e60921. *Joint first authors; #Joint senior authors
10	WOS:000313812100034	Article	Montonen, J., Boeing, H., Fritsche, A., Schleicher, E., Joost, H.G., Schulze, M.B., Steffen, A., **Pischon, T.** Consumption of red meat and whole-grain bread in relation to biomarkers of obesity, inflammation, glucose metabolism and oxidative stress. Eur J Nutr 52(1): 337-345, 2013.

Source: ISI Web of Science; As of 14.04.2014

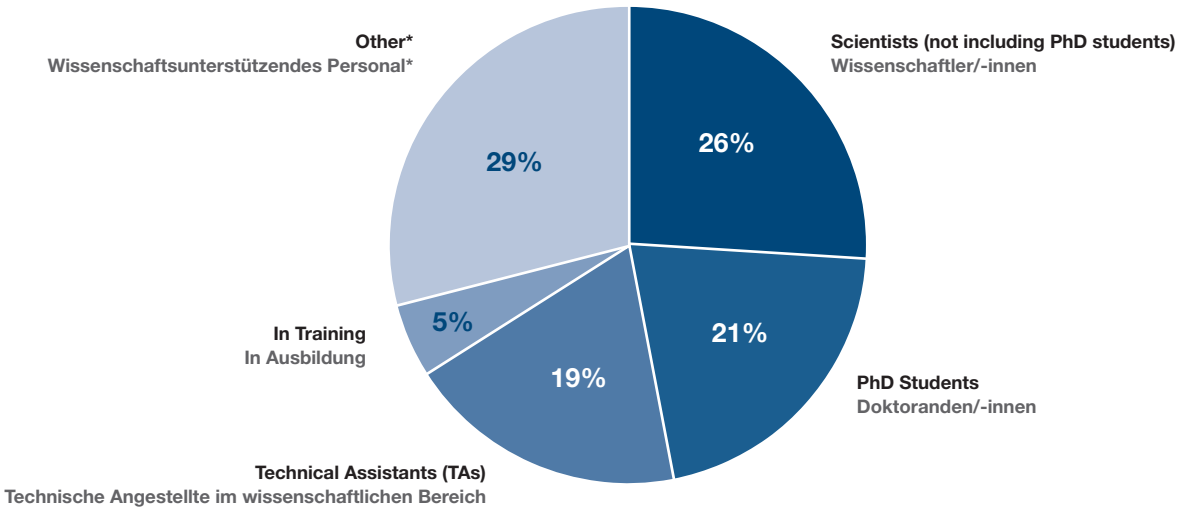
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Personnel (as of December 31, 2013)
Personalstand in Köpfen (Stichtag 31.12.2013)

	Total Gesamt	limited contract befristet	permanent unbefristet	in-house financed grund-finanziert	third-party financed drittmittel-finanziert
Scientists (not including PhD students) Wissenschaftler/-innen	286	229	57	194	92
PhD Students Doktoranden/-innen	237	237	0	128	109
Technical Assistants (TAs) Technische Angestellte im wissenschaftlichen Bereich	218	100	118	180	38
In Training In Ausbildung	60	60	0	60	0
Other* Wissenschaftsunterstützendes Personal*	323	112	211	303	20
SUMME	**1.124**	**738**	**386**	**865**	**259**

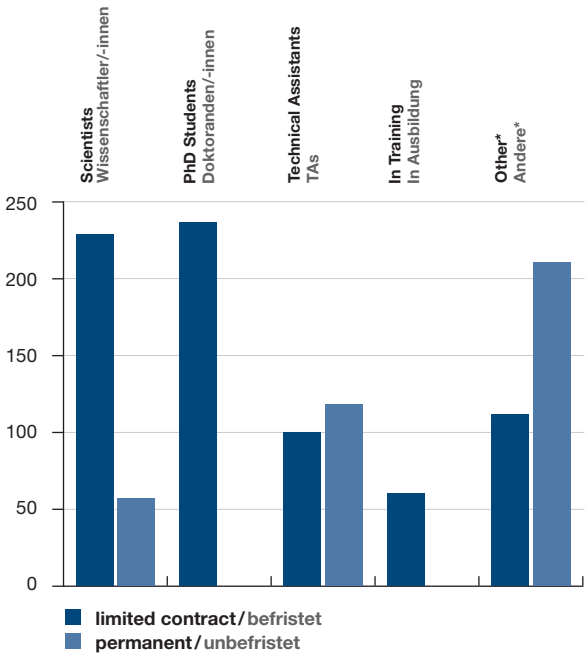


* Infrastructure, administration, technology transfer, DZHK grant management at the MDC, BIH management

Type of Employment Contract
Art der Verträge

	limited contract befristet	permanent unbefristet
Scientists Wissenschaftler/-innen	229	57
PhD Students Doktoranden/-innen	237	0
Technical Assistants TAs	100	118
In Training In Ausbildung	60	0
Other* Andere*	112	211
SUMME	**738**	**386**

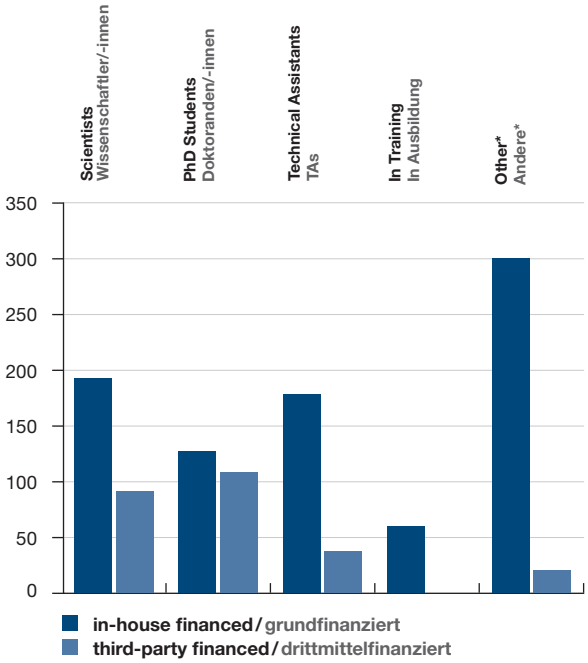
* Infrastructure, administration, technology transfer, DZHK grant management at the MDC, BIH management



Type of Financing
Art der Finanzierung

	in-house financed grundfinanziert	third-party financed drittmittel-finanziert
Scientists Wissenschaftler/-innen	194	92
PhD Students Doktoranden/-innen	128	109
Technical Assistants TAs	180	38
In Training In Ausbildung	60	0
Other* Andere*	303	20
SUMME	**865**	**259**

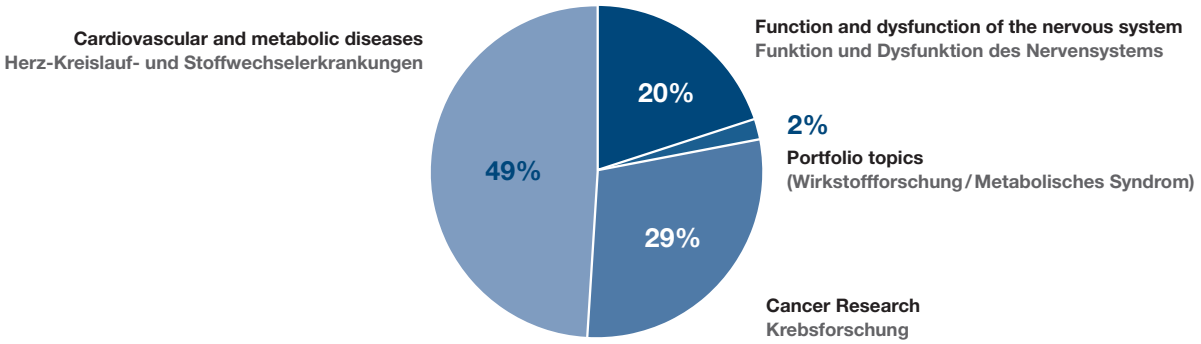
* Infrastructure, administration, technology transfer, DZHK grant management at the MDC, BIH management



Financing / Finanzierung

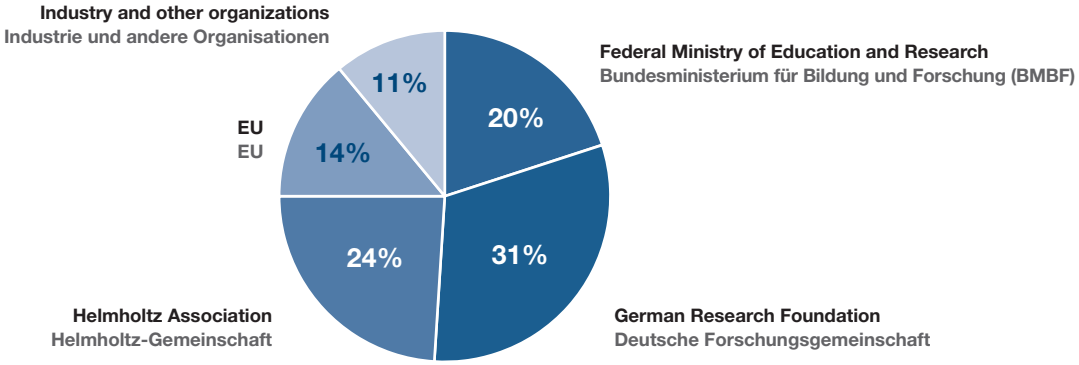
Costs of research programs in 2012 in TEUR
Kosten der Forschungsprogramme in 2012 in TEUR (Vollkosten)

Programs & Categories / Programme und Kategorien	Costs / Kosten
Cancer Research / Krebsforschung	16.499
Cardiovascular and metabolic diseases / Herz-Kreislauf- und Stoffwechselerkrankungen	27.473
Function and dysfunction of the nervous system / Funktion und Dysfunktion des Nervensystems	10.960
Portfolio topics (Wirkstoffforschung / Metabolisches Syndrom)	924
Total Program Costs / Gesamt	**55.856**



Extramural funding in 2012 in TEUR
Drittmittelfinanzierung 2012 in TEUR

Extramural funds / Drittmittelgeber	Amounts / Drittmittelausgaben
Federal Ministry of Education and Research / Bundesministerium für Bildung und Forschung (BMBF)	4.207
German Research Foundation / Deutsche Forschungsgemeinschaft	6.738
Helmholtz Association / Helmholtz-Gemeinschaft	5.167
EU / EU	3.014
Industry and other organizations / Industrie und andere Organisationen	2.382
Total Program Costs / Gesamt	**21.508**



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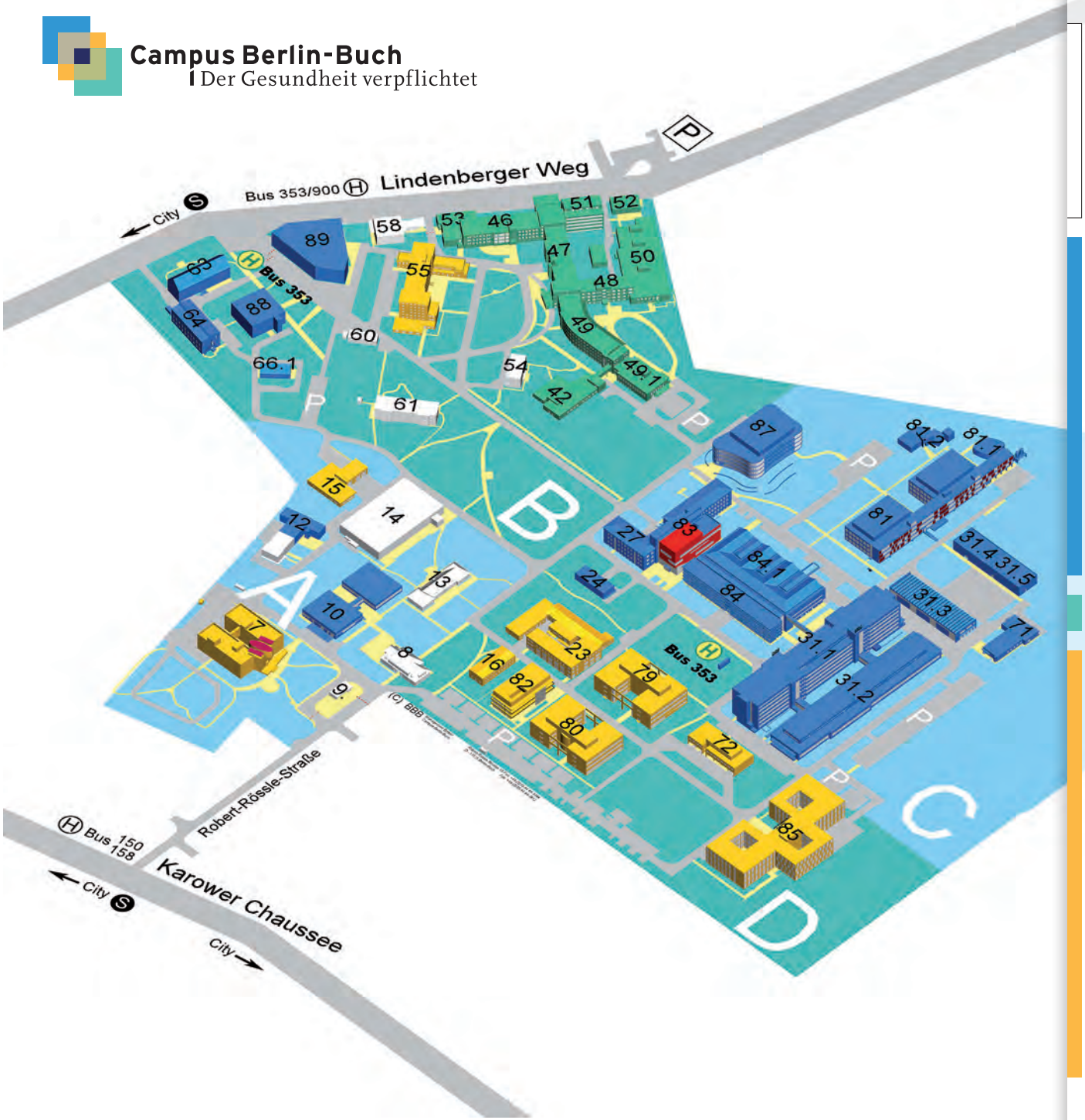
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Campus Berlin-Buch
Der Gesundheit verpflichtet



Common Facilities

A 8	Gate House with Café Max and apartments
A 9	Reception gate
A 13	Gläsernes Labor / Life Science Learning Lab; Campus Info Center
A 14	Cafeteria

MDC Guesthouses

B 54	Hans Gummel House
B 61	Salvadore Luria House with kindergarden

Research

Max Delbrück Center for Molecular Medicine (MDC)

C 27	Walter Friedrich House
C 31.1 -2	Max Delbrück House
C 31.3 -5	Energy and Infrastructure Center South (EZS)
C 83	MDC.C / Max Delbrück Communications Center
C 84	Hermann von Helmholtz House
B 63, 64	Research Services
B 88	Ultrahigh Field Facility
B 89	Max Rubner House
A10	Library

Leibniz-Institut für Molekulare Pharmakologie (FMP)

C 81	FMP

Facilities shared by MDC and FMP

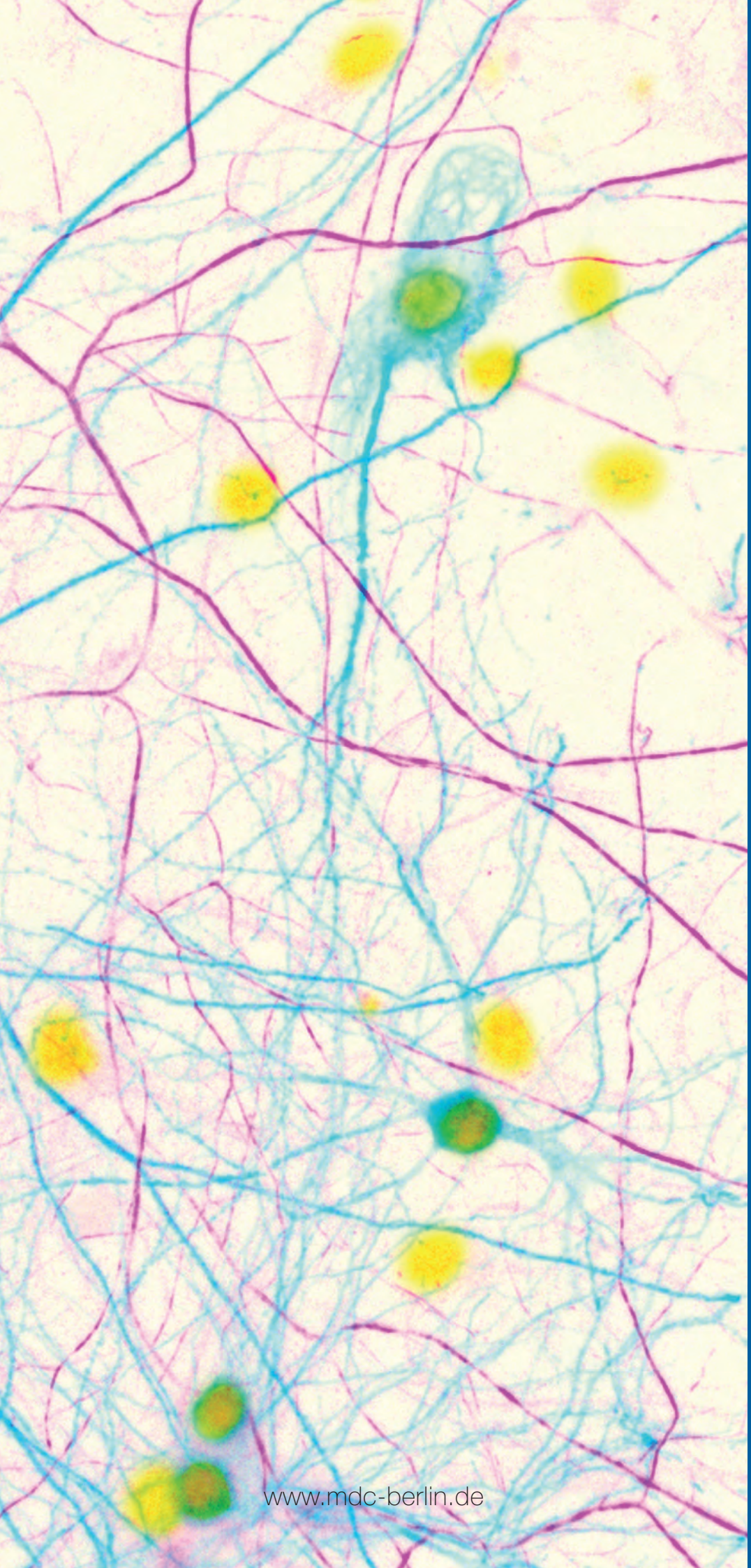
C87	Timoféeff-Ressovsky-Haus

Clinical Research

B 42-53	Experimental and Clinical Research Center (ECRC)

Companies

A 7	Eckert & Ziegler AG, Eckert Wagniskapital und Frühphasenfinanzierung (EWK)
A 15	Car mechanics, EZAG, Gardener
B 55	**OCVH - Oskar-und-Cécile-Vogt-Haus** BBB-post office, 8sens.biognostic, FILT, MRI, ConGen, E.R.D.E., ART-CHEM, LIPIDOMIX, Roboklon, Myelo, Fresenius, Patent lawyers Dr. Baumbach, L.O.T., WISE, Octreopharm, Institut E & G, NIKON, Hermes-Analytik, Steinbeis-Transfer-Institut, I.M.S.M., AJ Innuscreen
B 64	epo
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