Research Report 2010

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FOR MOLECULAR MEDICINE
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Research Report 2010
(covers the period 2008-2009)

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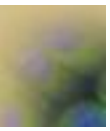

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Structural and Functional Genomics

Tumor Immunology

Function and Dysfunction of the Nervous System
Funktion und Dysfunktion des Nervensystems

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Einleitung

Prof. Dr. Walter Rosenthal

Building our future on existing strengths: Extending the path from basic science to clinical medicine

Like living things, scientific institutions need to adapt to changes in their environment; along the way, they help to reshape it. This process is particularly important in times of dramatic change, which is the situation for the biomedical sciences: They are experiencing very rapid technological and conceptual progress against a backdrop of shifting economic and social conditions. This is having a direct, significant impact on the way our laboratories work and our pursuit of the MDC's primary mission: to connect fundamental discoveries in the life sciences to the field of human health. The impact of new technologies and newly gained insights into basic biological processes can be seen in the reports of our scientific and clinical groups and facilities, which form the core of this document and aim to give colleagues, supporters, and many others an overview of our accomplishments during 2008 and 2009. But this is not the whole story of what has been happening over the past two years

Den Weg von der Grundlagenforschung in die klinische Medizin ausbauen: Entwicklung und Stärkung des vorhandenen Potentials

Eine wissenschaftliche Institution muss sich genau so wie ein lebender Organismus ständig an neue Umweltbedingungen anpassen und diese gleichzeitig aktiv beeinflussen. In den Lebenswissenschaften erleben wir gegenwärtig große und rasante Veränderungen. Sie haben tiefgreifende Auswirkungen auf die Arbeitsweise unserer Forschungsgruppen bei einer unserer wichtigsten Aufgabe: der Übertragung fundamentaler molekularbiologischer Erkenntnisse in die klinische Anwendung. Der Schwerpunkt des hier vorliegenden Berichtes ist der Überblick über die in den Jahren 2008 bis 2009 erreichten wissenschaftlichen Ergebnisse – für unsere Kollegen, unsere Förderer und die Öffentlichkeit.

Die Bedeutung neuer Erkenntnisse aus der Grundlagenforschung wird durch die Berichte der einzelnen Forschungsgruppen herausgestellt. Unsere Forschungsergebnisse sind jedoch nicht der alleinige Inhalt dieses Berichts. Es geht um mehr. So geht es auch um die Rolle

at the MDC. The role of the institute is to provide a framework in which researchers can carry out their work at the highest possible level and, ideally, to translate their findings into new forms of prevention, diagnoses and therapies of diseases. To accomplish these ambitious goals we must respond to changes and anticipate new opportunities – which, in the current landscape of research, means reworking some of our internal infrastructures, as well as those that connect us to our partners in Berlin, Germany, and the international scientific community. In this brief introduction I would like to give readers a glimpse of the logic behind our current activities and future plans.

Several of the major, ongoing projects were conceived and began maturing during the administration of my predecessor, Walter Birchmeier, who served as the Scientific Director of the MDC until the end of 2008 and continues to head a research group within the institute. When I had the privilege of being named his successor at the beginning of 2009, I stepped into an institute that is thriving and producing a steady output of excellent scientific work. That state of affairs should be considered part of Walter Birchmeier's legacy. All of us who have worked with him know of the high priority he has placed on the quality of the MDC's research and the continual need to recruit talented young scientists from around the world. The success of that strategy is documented within these pages.

Research reports provide an institute-centric overview of the state of the art in the main areas of our research: cardiovascular and metabolic disease, cancer, and function and diseases of the nervous system. This work is going on at a time when the life sciences are becoming very integrative. Over the last decade, advanced new methods have produced amazing amounts of data which cover aspects of life that have never been directly observable and are finally allowing us to link the structures and functions of molecules to the behavior of cells, embryonic development, and the processes that underlie life-threatening diseases. For example, until very recently, it was virtually impossible to capture quantitative data about the proteins active in different types of cells – a huge bottleneck in molecular biology.

des MDC als Netzwerk und Infrastruktur, das die Interaktion der Wissenschaftler aus den unterschiedlichen Disziplinen untereinander fördert und den Wissenschaftlern modernste Technologien und Infrastrukturen für die Entwicklung neuer Methoden nicht nur in der Grundlagenforschung, sondern auch in der Prävention, Diagnostik und Therapie von Krankheiten zur Verfügung stellt.

Um dieses anspruchsvolle Ziel zu erreichen, müssen wir auch unsere wissenschaftliche und organisatorische Infrastruktur nachjustieren. Ebenso müssen wir unsere Verflechtung mit den wissenschaftlichen Partnern speziell in Berlin, in ganz Deutschland und in der internationalen wissenschaftlichen Gemeinschaft weiter entwickeln.

Mit dieser kurzen Einführung möchte ich kurz das Konzept unserer gegenwärtigen Arbeit und der zukünftigen Entwicklung des MDC skizzieren. Es wurde unter der Leitung meines Vorgängers, Walter Birchmeier, entworfen. Anfang 2009 hatte ich die Ehre, seine Nachfolge als Direktor des MDC anzutreten, während Walter Birchmeier sich wieder ganz der wissenschaftlichen Arbeit als Leiter seiner Forschungsgruppe widmete. Ich übernahm von ihm die Leitung eines Institutes, dessen Mitarbeiter kontinuierlich wichtige wissenschaftliche Erkenntnisse gewinnen und in den besten Zeitschriften veröffentlichen. Diese Leistung kann man als Vermächtnis Birchmeiers an die nachfolgende jüngere Generation ansehen: Wir alle haben in der Zusammenarbeit mit ihm erlebt, mit welcher Beharrlichkeit er die für das MDC wichtigsten Ziele verfolgte: die Erhöhung der wissenschaftlichen Qualität und die Rekrutierung begabter junger Forscher aus der ganzen Welt. Dieser Bericht dokumentiert auch den Erfolg seiner Arbeit als Direktor des MDC.

In den nachfolgenden Forschungsberichten werden die gewonnenen wissenschaftlichen Erkenntnisse in unseren Hauptarbeitsgebieten kardiovaskuläre und metabolische Erkrankungen, Krebs sowie Erkrankungen des Nervensystems dargestellt. Wie Sie in den einzelnen Berichten feststellen können, gibt es am MDC aber viele „Grenzgänger"; denn molekulare Fragestellungen lassen sich häufig nicht auf einzelne Organe oder Krankheiten beschränken.

Darüber hinaus erleben wir gegenwärtig eine rasante Entwicklung bei den verfügbaren Technologien und

That barrier is now falling thanks to methods such as SILAC, a specialty of the laboratory of new group leader Matthias Selbach. His lab is intensively collaborating with several others, including that of Nikolaus Rajewsky, who has modified the method to investigate the role of various types of small, noncoding RNAs in a range of developmental and cellular processes.

Another example of transcending barriers is the search for multiple genes that contribute to complex health problems such as cardiovascular disease, Alzheimer's, and many types of cancer. For many years it has been clear that some forms of these diseases have a genetic component, but the patterns by which they are inherited are obscure. Identifying the genes that are responsible will likely require a huge effort involving many laboratories and clinics, very large groups of patients and their families, new animal models, and sophisticated computer algorithms. The MDC will play an active role in the establishment of a huge national cohort organized by the Helmholtz Association in Germany, with the participation of hundreds of thousands of randomly selected subjects from all over the country, in a major project involving Helmholtz partners such as the DKFZ, the Helmholtz Center for Health and the Environment (HMGU) in Münich, the Helmholtz Center for Infectious Diseases (HZI) in Braunschweig, the German Center for Neurodegenerative Diseases (DZNE) in Bonn, and other national partner institutes. Regionally, the MDC will cooperate with the Charité-Universitätsmedizin in Berlin, the German Institute for Nutritional Research (DIfE) in Potstdam and the Robert Koch Institute (RKI) in the center of Berlin to recruit patients from three regions within Berlin-Brandenburg: on the Charité Benjamin Franklin campus in the southwest, on the Charité Campus in the city center, and on the Berlin-Buch campus in the northeast. Locally, a total of 40,000 patients will be recruited. These people and the rest of the cohort will be systematically studied and the results will provide a basis for epidemiological research into the causes of diabetes, cardiovascular diseases, neurodegenerative diseases and cancer.

Yet even prior to the establishment of this important resource, our groups have made significant progress

Verfahren, welche die Lebenswissenschaften insgesamt nachhaltig beeinflussen. Die Hochdurchsatzverfahren, die in kurzer Zeit eine riesige Menge an Daten liefern, die ein Jahrzehnt zuvor nicht vorstellbar waren, eröffnen nicht nur ungeahnte Möglichkeiten, sondern stellen die Wissenschaftler auch vor neue Herausforderungen. Sie erlauben die komplette Bestandsaufnahme einer Zelle, Organ, Organismus zu einem bestimmten Zeitpunkt auf Genom-, Transkriptom-, Proteom- und Metabolomebene. Mit Hilfe eines umfangreichen ausgeklügelten Datenmanagements und -analyse und der Entwicklung mathematischer Modelle können wir zukünftig eine Gesamtschau komplexer Prozesse erhalten, wie Embryonalentwicklung oder die Entstehung komplexer Krankheiten. Ein wichtiger Baustein ist dabei weiterhin die Aufklärung der Funktion einzelner Proteine in verschiedenen Zelltypen. Hochdurchsatzverfahren erlauben auch hier jetzt eine Übersicht der Auswirkungen auf das Gesamtsystem – eine Möglichkeit, die der Molekularbiologie bisher weitgehend verschlossen war. Ein wichtiger Beitrag ist die Weiterentwicklung der sogenannten SILAC-Methode durch unseren in 2007 hinzu gekommenen Nachwuchsgruppenleiter Matthias Selbach. Seine Gruppe kooperiert z.B. intensiv mit Nikolaus Rajewsky. Die Anwendnung des weiterentwickelten Verfahrens im Rahmen eines gemeinsamen Projektes hat entscheidend zum Verständnis der Funktion verschiedener Typen kleiner nicht kodierender RNS-Moleküle beigetragen.

Seit vielen Jahren ist es klar, dass die meisten Krankheiten komplexe genetische und umweltbedingte Ursachen haben. Daher wird die Helmholtz-Gemeinschaft gemeinsam mit Universitäten und außeruniversitären Forschungseinrichtungen in den nächsten Jahren eine große nationale Kohorte aufbauen. Nach einer dreijährigen Planungs- und Pilotphase sollen 200.000 Studienteilnehmer aus verschiedenen Regionen Deutschlands rekrutiert, medizinisch untersucht und systematisch weiter beobachtet werden. Die gewonnenen Daten dienen als Basis für zukünftige epidemiologische Untersuchungen zu den Ursachen von Diabetes, Herz-Kreislauf-Erkrankungen, neurodegenerativen Erkrankungen und Krebs. Das MDC wird an der Rekrutierung der Großkohorte von 200.000 Probanden aktiv teilnehmen. Dies ist ein wichtiges Kooperationsprojekt mit den anderen Gesundheitszentren innerhalb der Helmholtz-Gemeinschaft, dem Deutschen Krebsforschungszen-

in discovering genes that contribute to these disease types. The groups of Norbert Hübner and Friedrich Luft, in a collaboration with Jan Monti of the Helios clinic, discovered that a molecule called Ephx2 plays a role in the development of arrhythmic beating of the heart and to sudden death through heart failure in hypertensive rats. The groups also provide evidence that Ephx2 plays a similar role in patients. This work benefited enormously from Hübner's studies of the rat, one of the most important model organisms in medical research, but which has lagged behind because of a lack of tools for genetic manipulation which are so highly developed in the mouse and other laboratory animals. The completion of the rat genome in 2004 has given a major boost to laboratories which combine computational and "wet lab" methods in the search for genes related to disease. New tools for the genetic manipulation of rats are the aim of the international, EU-funded consortium EURATools, which is coordinated by Norbert Hübner.

This type of work demonstrates a general principle that has been successful at the MDC: combining studies of fundamental mechanisms and model organisms with work on patients. Thomas Willnow's studies of the family of low density lipoprotein receptor-related proteins (LRP) led to the discovery of a mechanism involving sorLA, a receptor expressed in neurons, and the accumulation of amyloid plaques – proteins which aggregate between nerve cells in the development of Alzheimer's disease. Systematic work on ion channels and transporters from the lab of Thomas Jentsch, who holds a joint appointment from the MDC and FMP, has revealed several mutations which cause changes in the structure of the inner ear and lead to congenital deafness.

The interface between clinical groups and basic research laboratories steadily produces findings that are having a direct impact on the diagnosis and treatment of disease. Over the past two years Peter Schlag, who has a joint appointment at the Charité and MDC, has established a Charité Comprehensive Cancer Center in downtown Berlin to ensure a coordinated approach to diagnoses, therapies, and the medical, psychological, and social care of patients. At the same time, Schlag's group at the MDC has identified the

trum (DKFZ) in Heidelberg, dem Helmholtz Zentrum München für Gesundheit und Umwelt (HMGU), dem Helmholtz Zentrum für Infektionsforschung (HZI) in Braunschweig und dem Deutschen Zentrum für Neurodegenerative Erkrankungen (DZNE) in Bonn sowie anderen nationalen Partnerinstituten. Regional wird das MDC in Zusammenarbeit mit der Charité – Universitätsmedizin Berlin, dem Deutschen Institut für Ernährungsforschung (DIfE) in Potsdam und dem Robert Koch-Institut (RKI) in Berlin-Mitte an drei Orten im Großraum Berlin-Brandenburg Probanden rekrutieren: im Südwesten am Charité-Campus Benjamin Franklin, im Stadtzentrum am Campus Charité Mitte und im Nordosten am Campus Berlin-Buch. Insgesamt sollen 40.000 Probanden rekrutiert werden.

Wie die nachfolgenden Beispiele zeigen, ist die am MDC betriebene molekularmedizinische Forschung die unverzichtbare Basis und häufig der Ausgangspunkt für Patienten- und Probanden-orientierte Projekte. Die Forschungsgruppen von Norbert Hübner und Friedrich Luft, in Zusammenarbeit mit Jan Monti, Helios-Klinikum in Berlin-Buch, konnten im Tiermodell der hypertensiven Ratte mit dem sogenannten Ephx2-Molekül und in „knockout"-Mäusen einen Faktor identifizieren, der für die Entstehung einer Herzinsuffizienz verantwortlich ist. Darüber hinaus konnten Hinweise dafür gefunden werden, dass Ephx2 eine ähnliche Rolle bei Patienten spielt. Dies ist ein gutes Beispiel für die Bedeutung von Krankheitsmodellen im Tier. Durch die Fertigstellung des Rattengenom-Projekts im Jahre 2004, an dem Hübners Gruppe maßgeblich beteiligt war, können genetische Veränderungen bei erkrankten Tieren leichter identifiziert werden. Heute koordiniert Hübner das von der EU geförderte Konsortium EURATOOLS, in dem wichtige neue Methoden der genetischen Analyse der Ratte entwickelt werden.

Erfolge dieser Art sind ein Ergebnis eines allgemeinen Arbeitsprinzips des MDC: der Kombination von Grundlagenforschung in Zellen und an Modellorganismen mit der Analyse von klinischen Daten. So führten Thomas Willnows Untersuchungen der LRP-Rezeptorfamilie zur Entdeckung eines Mechanismus für die Akkumulation von Amyloidplaques, wie sie in den Nervenzellen bei der Alzheimer-Krankheit eine Rolle spielen. An diesem Vorgang ist sorLA, ein Rezeptor dieser Familie, der in Neuronen exprimiert wird, ursächlich beteiligt.

protein MACC1 as a marker for metastatic cells in colorectal cancer (CRC). MACC1 is involved in regulating injury response and tissue growth in the HGF/MET signaling pathway, which has been intensively studied in Walter Birchmeier's lab. The finding should allow doctors to assess the risk that CRC in a patient will develop into a dangerous metastatic form, which has important implications in designing an effective, individualized treatment for the disease. Additionally, it establishes a mechanistic link between tumors and a crucial pathway involved in human development, contributing to our understanding of "cancer stem cells."

Yet another example of this type of interface is the long-term collaboration of the labs of Claus Scheidereit (MDC) and Bernd Dörken (Charité/MDC), along with the group of Martin Lipp and many other colleagues. They have discovered many links between the behavior of the transcription factor NF-kB and the development of Hodgkin's disease and other lymphomas. Here, too, the findings are exposing mechanisms that should result in improved diagnosis and treatment for cancer patients.

Another important step in carrying out our mission is to ensure that discoveries are capitalized on; to develop a small-molecule inhibitor into a drug, for example, requires years of work and significant investments, and often the "ball is fumbled" in the handoff from basic science to industry. Even a very important finding may require many further steps of development before it is at a state that it has become a basis for founding a new company or before it attracts the interest of the pharmaceutical industry. To smooth the way, the BMBF has sponsored a competitive program called Go-Bio, which provides several years of support to academic groups to bridge the gap between a basic science discovery and commercialization. Since some promising projects are not quite at a state to apply, the MDC has developed an internal stimulus program called "pre-Go-Bio," to which groups can apply for three-year funding to prepare for an application within the Go-Bio program or within another program with a similar intention. In 2008 two groups were awarded pre-Go-Bio grants; two more received funding under the program in 2009.

Im Labor von Thomas Jentsch werden die Funktion und die Auswirkungen einer Fehlfunktion von Ionenkanälen und -transportern erforscht. Dabei entdeckten die Wissenschaftler mehrere Mutationen in bestimmten Ionenkanälen, die für Veränderungen in der Struktur des Innenohrs verantwortlich sind und zu angeborener Taubheit führen. Thomas Jentsch gehört gleichzeitig zu unserem Nachbarinstitut, dem Leibniz-Institut für Molekulare Pharmakologie (FMP). Die Forschungsgruppe wird von beiden Instituten jeweils zur Hälfte finanziert. Eine Besonderheit, die die enge Zusammenarbeit zwischen FMP und MDC illustriert.

Ein wichtiges Anliegen des MDC ist und bleibt die enge Interaktion mit klinischen Gruppen der Charité. Die bei uns auf dem Campus Berlin-Buch gemeinsam mit der Charité entwickelten Schnittstellen zwischen klinischen Gruppen und experimentell orientierten Grundlagenwissenschaftlern ermöglicht nachhaltige Auswirkungen auf die Diagnostik und Behandlung von Krankheiten. In den vergangenen zwei Jahren hat der Charité-Kliniker Peter Schlag, der auch Leiter einer Arbeitsgruppe am MDC ist, im Zentrum von Berlin das sog. Charité Comprehensive Cancer Center aufgebaut. Dieses Zentrum ermöglicht ein koordiniertes Herangehen an diagnostische, therapeutische, psycho- und sozialmedizinische Probleme der Patienten. Im Rahmen ihrer experimentell-wissenschaftlichen Arbeit am MDC hat die Gruppe von P. Schlag in Zusammenarbeit mit Walter Birchmeier das Protein MACC1 identifiziert, das als diagnostischer Marker für Dickdarm- und Rektumkrebs verwendet werden könnte. MACC1 spielt im HFG/MET-Signalweg, der von Walter Birchmeiers Gruppe intensiv untersucht wird, eine wichtige Rolle und reguliert dadurch Gewebswachstum MACC1 und kann in der Klinik bei der Abschätzung des Risikos einer metatstatischen Ausbreitung des Krebses wertvolle Informationen liefern, die in Zukunft möglicherweise eine gezielte, individuell angepasste Therapie ermöglicht. MACC1 spielt möglicherweise auch eine Rolle bei der Entstehung sogenannter Krebsstammzellen.

Ein weiteres Ergebnis dieser Art von interdisziplinärer Forschung liefert die seit langem bestehende Zusammenarbeit der Forschungsgruppen von Claus Scheidereit (MDC) und Bernd Dörken (Charité/MDC) sowie der Gruppe von Martin Lipp (MDC) und weiteren Kollegen. Sie haben zahlreiche Bindeglieder zwischen dem

The activities described above – and dozens of others found within this report – demonstrate that the MDC's approach to translational research has produced results that are having an impact on the practice of medicine. Yet there is much more to be done. Molecular biology and medicine arise from different traditions, their practitioners are still trained in quite different ways, and radically different styles of work make it difficult for a single scientist to be equally at home in both worlds. As a result, there is not enough communication between the complementary perspectives of a researcher working on basic mechanisms and a physician who has to gain a holistic understanding of the difference between a healthy person and someone suffering from a disease. One solution, sought by many of our colleagues, is to establish long-term partnerships between fundamental research laboratories and clinical groups. But we believe that the significant "culture gap" that still exists needs to be bridged in a systematic, practical, and effective way. These are the aims of several major, ongoing initiatives that we regard as crucial to our future and which I will briefly describe in the next sections. Each of the projects is explored in more depth later in the report.

The Experimental and Clinical Research Center (ECRC)

Since the founding of the MDC, interactions between basic and clinical research groups have been strongly supported through a variety of mechanisms. Building on existing strengths, the Experimental and Clinical Research Center (ECRC), a collaborative project with the Charité-University Medical School, was established in 2007 on the Berlin-Buch campus, to foster translational research activities. Our close partnership with the Charité means that it will be possible for ECRC projects to extend to all patients who are being cared for anywhere within the university hospital system. Thus the 120,000 stationary patients and 600,000 who are being treated in out-patient clinics represent an important potential pool for biomedical studies. Details of the ongoing activities within the ECRC are presented later in the book; here I will briefly describe the ongoing activities.

Transkriptionsfaktor NF-κB, der Entstehung des Hodgkin-Lymphoms und anderer Lymphome aufgeklärt. Auch hier sollten sich neue Diagnose- und Therapieverfahren zum Nutzen betroffener Patienten ergeben.

Von hoher Priorität ist auch das Bemühen um die wirtschaftliche Verwertung unserer Forschungsergebnisse. Die Entwicklung einer niedermolekularen interaktiven Substanz bis zu einem einsatzfähigen Medikament kann viele Jahre dauern und erfordert erhebliche Investitionen. Leicht wird dabei das notwendige „Passspiel" zwischen Wissenschaft und Industrie „verdribbelt". Selbst vielversprechende Entwicklungen bedürfen intensiver weiterer Arbeit, bevor ein Reifegrad erreicht wird, der die Gründung eines neuen Unternehmens rechtfertigen oder das Interesse der pharmazeutischen Industrie wecken kann. Zur Unterstützung dieses Prozesses hat das BMBF das Wettbewerbsprogramm Go-Bio ausgeschrieben, das akademischen Gruppen mehrjährige Unterstützung zur Überbrückung des Grabens zwischen Grundlagenforschung und kommerzieller Anwendung ermöglicht. Auch im MDC gibt es vielversprechende Projekte, die jedoch teilweise noch nicht für eine Bewerbung geeignet sind. Daher hat das MDC ein internes Förderprogramm „pre-Go-Bio" aufgelegt, bei dem sich Wissenschaftler um eine dreijährige Finanzierung zur Vorbereitung eines Go-Bio-Antrages oder einer analogen Förderung bewerben können. Bisher werden 4 Projekte gefördert.

Die hier im Bericht dargestellten Ergebnisse zeigen eine Vielzahl weiterer anwendungsnaher Vorhaben. Sie sind das bisherige Ergebnis der Zusammenarbeit von Grundlagenwissenschaftlern und Klinikern. Selbstverständlich bleibt noch sehr viel zu tun. Molekularbiologie und Medizin haben sehr verschiedene Wissenschaftstraditionslinien. Die jeweiligen Spezialisten sind sehr unterschiedlich ausgebildet, und sie folgen oft einer sehr unterschiedlichen Arbeitsmethodik. Daher ist es für einen Wissenschaftler nach wie vor oft sehr schwer, in beiden Feldern gleichermaßen zu Hause zu sein.

Dies bedeutet für uns, dass die Kommunikation zwischen einem experimentellen Grundlagenwissenschaftler wie einem Biologen oder Biochemiker, der am Verständnis der Grundmechanismen des Lebens arbeitet, und einem Kliniker, der ein ganzheitliches Ver-

The ECRC offers research space and funding, on a competitive basis, for research projects of the Charité and the MDC aiming to develop new methods for disease prevention, diagnosis, and therapy. It provides access to out-patient clinics and an infrastructure for investigator-initiated studies (phase 1 and early phase 2). For clinicians, the ECRC offers protected research time outside the constraints of clinical routines in the hospital, and close proximity to MDC research groups.

Members of all the clinical departments of the Charité and MDC scientists working on relevant projects are eligible to participate in ECRC activities, upon the submission of a proposal and a positive evaluation through peer review. Ongoing projects cover all the major research areas of the MDC (cancer, cardiovascular disease, and diseases of the nervous system).

The ECRC's educational objectives address the fact that physicians and biologists are still trained in quite different ways and new programs are necessary to train the "physician-scientists" of the future.

In brief, the key features of the ECRC are the following:

- Clinicians or basic researchers can apply for independent project groups to conduct a translational project within the ECRC. Funding is provided for three years (extension possible). The funding descision is based on a competitive evaluation involving external experts.
- Clinicians and researchers of the MDC can jointly submit proposals for collaborative projects which can be funded through the ECRC after a competitive evaluation (KKP program). The projects are conducted at the ECRC and/or the MDC.
- Within the ECRC, the MDC and Charité jointly sponsor a training program for clinicians (KAP) in basic molecular biology, in which clinicians take a break from their duties and join one of the MDC's laboratories. This promotes long-term collaborations between the home and host .
- Clinicians direct out-patient clinics and carry out clinical studies within the ECRC;
- Clinicians and scientists have access to clincical research center (outpatients clinics, beds for clin-

ständnis für den gesunden und den kranken Organismus entwickeln muss, nicht so einfach zu bewerkstelligen ist. Eine Lösungsmöglichkeit besteht in der Einrichtung von Forschungsgruppen, in denen Grundlagenwissenschaftler und Kliniker langfristig eng zusammenarbeiten. Gleichwohl bleibt ein „kultureller Graben" zwischen beiden Welten bestehen, der jedoch auf systematische, pragmatische und effektive Weise überwunden werden kann. Dies ist die Motivation für mehrere größere langfristige Initiativen, die wir für die Zukunft des MDC für entscheidend halten. Ich werde sie hier nur kurz skizzieren, ohne einer späteren genaueren Beschreibung im Bericht vorzugreifen.

Das Experimentelle und Klinische Forschungszentrum (ECRC)

Seit Gründung des MDC hat die Zusammenarbeit von Grundlagenforschung und klinischer Medizin durch eine Serie von Maßnahmen intensive Förderung erfahren. Darauf baut das ECRC auf, bei dem es sich um ein kooperatives Projekt mit der Charité-Universitätsmedizin handelt, das 2007 auf dem Bucher Campus gegründet wurde, um die Translationsforschung zu unterstützen. Diese enge Partnerschaft mit der Charité ermöglicht es, dass zukünftige ECRC-Projekte Probanden und Patienten tendenziell aus allen Bereichen der Universitätskliniken rekrutieren können. Die Kliniken behandeln etwa 120 000 Patienten stationär und betreuen ca. 600 000 Personen ambulant. Dies ist ein bedeutendes Hintergrundpotential für ambitiöse Projekte.

Es folgt eine kurze Beschreibung der laufenden Projekte.

Das ECRC bietet Forschungsinfrakstruktur und Finanzierungsmöglichkeiten, für die sich Forschungsprojekte der Charité und des MDC mit dem Ziel der Entwicklung neuer Methoden der Vorbeugung, Diagnostik und Therapie von wichtigen Krankheiten bewerben können. Hinzu kommt als Infrastruktur die Möglichkeit, Probanden ohne Klinikaufnahme zu rekrutieren und Phase-1- und frühe-Phase-2-Studien zu betreiben. Für klinische Forschung im engeren Sinne bietet das ECRC die Möglichkeit, ärztlich kontrollierte Forschung jenseits der engen Rahmenbedingungen und Abläufe im Krankenhaus und in enger räumlicher Nachbarschaft zu Forschergruppen auf dem MDC-Campus durchzuführen.

cical trials) and technology platforms, (e.g. ultra-high-field MR instruments, GMP facility, bio-banking).

Currently, the ECRC consists of two buildings on the Berlin-Buch campus: the Research Building ("Forschungshaus") of the Charité (former Robert-Rössle-Klinik) for clinical activities and a new building of the MDC for the ultrahigh-Field MR facility equipped with 7 T and 3 T whole-body human MR scanners and a 9.4 T animal MR scanner. Constructions for a third building financed by the MDC, comprising 2600 m^2 of laboratory and office space, will begin in spring 2010, with a target completion date of 2012.

A big step forward was the appointment of Thoralf Niendorf as group leader at the MDC, as director of the ultrahigh-Field MR facility and professor (W3, chair) for ultrahigh-Field MR at the Charité in August 2009. A physicist by training, Thoralf Niendorf is a leading expert in MR imaging with a research experience in academia and industry.

The ECRC structure will be crucial in establishing and utilizing the Helmholtz Cohort, in which 200,000 people will be recruited across Germany, including 40,000 from the Berlin region.. We are currently recruiting for a W3 professorship (chair) in epidemiology. Besides other research projects, the appointed scientist will administer the Berlin arm of the cohort.

The Berlin Institute for Medical Systems Biology (BIMSB)

The greatest challenge for today's biomedical science is to take what we have learned about the discrete elements of biological systems – such as genes, RNAs, and proteins – and understand how they work together in extremely complex, dynamic networks to produce higher levels of structure such as cells, tissues, and organisms. This integrative view is essential to learning how biological processes change during disease. Obtaining it will require amassing and analyzing huge amounts of data from high-throughput technology platforms, *in vitro* studies, work with cells, and genetic and developmental studies of complete organisms with an aim to drawing parallels between other species and humans. The narrowing gap

An den Forschungsprojekten des ECRC können im Prinzip je nach Fragestellung alle Wissenschaftler des MDC und aller beteiligten Abteilungen der Charité teilnehmen. Die laufenden Projekte konzentrieren sich auf die Hauptrichtungen des MDC, nämlich Krebsforschung, Herz-Kreislauf-Forschung und Krankheiten des Nervensystems. Entsprechende Abteilungen der Charité sind beteiligt.

Die Ausbildungspläne des ECRC konzentrieren sich auf die Überbrückung des immer noch sehr verschiedenen Ausbildungsganges von Ärzten und Biologen und zielen auf das Training eines klinisch-experimentell spezialisierten Nachwuchses für die Zukunft.

Kurz zusammengefasst lässt sich das ECRC wie folgt charakterisieren:

- *Kliniker oder Grundlagenforscher können sich zu unabhängigen Forschungsgruppen zusammenschließen, um ein Translationsprojekt durchzuführen. Finanzielle Förderung ist für drei Jahre vorgesehen, Verlängerung und Ausbau sind möglich. Der Zuschlag wird auf der Basis einer wettbewerblichen Ausschreibung vergeben.*
- *Kliniker oder Grundlagenforscher können kooperative Projekte zur Förderung einreichen, die ebenfalls auf interner Ausschreibungsbasis vergeben wird (KKP-Programm). Je nach Sachlage werden solche Projekte im ECRC oder in anderen Einheiten des MDC durchgeführt.*
- *Das ECRC führt ein Ausbildungsprogramm (KAP) über molekulare Grundlagen der Biomedizin durch, das von MDC und Charité-Kliniken gemeinsam unterstützt wird. So können klinisch tätige Ärzte für einige Zeit in MDC-Laboratorien arbeiten, wodurch zu langfristiger Zusammenarbeit von gastgebender Abeilung und Gastwissenschaftler motiviert wird.*
- *Das ECRC führt klinische und tagesklinische Forschungsarbeiten mit Patienten unter Anleitung von erfahrenen Klinikern durch.*
- *Sowohl klinische als auch experimentelle Forscher haben Zugriff zur klinischen Forschungskapazität (stationäre und tagesklinische Betreuung) und zur technischen Ausrüstung (z.B. ultrahochauflösende Magnetresonanz, GMP-Infrastruktur, Biobankauswertung).*

between disciplines such as biology, chemistry, physics, mathematics, engineering and other fields are dissolving to make this one of the most exciting eras in science, and a time at which progress can often only be made through a new type of interdisciplinarity. Our own history aptly reflects this: work carried out 50 years ago by the physicist Max Delbrück, after whom the MDC is named, with the geneticist Timoffeéf Ressovsky played a central role in the establishment of modern molecular biology. This trend is increasing and we must ensure that young scientists are given the tools and concepts to cope.

Over the past two years our reflections about these issues at the MDC have coalesced into a proposal for the establishment of a new institute, the Berlin Institute for Medical Systems Biology (BIMSB), as an important extension of our activities in the field. A solid scientific program has been established, and the proposed size and scientific program were very positively evaluated by an international panel of experts from relevant fields in April 2009. More details on the research and ongoing projects of the BIMSB appear later in the book. Here I will briefly report on the status of the project as a whole.

Support for the establishment of BIMSB comes through the BMBF Initiative "Innovation and leading research in the new German states" as well as by the Senate of Berlin. The MDC provides administration, infrastructure, and staff in support of the projects and new groups. Nikolaus Rajewsky played a central role in the development of the proposal and is the speaker and scientific coordinator of the project. As well as recruiting new groups with a systems biology orientation to the MDC, the project is implementing scientific collaborations with the Charité, the Humboldt University of Berlin (HU), the Free University of Berlin (FU), the Leibniz Institute for Molecular Pharmacology (FMP), the Max Planck Institute for Molecular Genetics, the MPI for Infection Biology, and other research institutions. In agreement with the partners of BIMSB, a site has been chosen in the city center for the construction of a BIMSB building: on the north campus of the HU Berlin close to its institute for theoretical biology, to Charité clinics, and the "Bernstein Center for Computational Neuro-

Gegenwärtig verfügt das ECRC auf dem Bucher Campus über zwei Gebäude: das Forschungshaus der Charité für klinische Forschung (vormals Robert-Rössle-Klinik) und das neue Gebäude, das das ultra-hochauflösende MR-Gerät mit 7 T und 3 T Ganzkörper MR Scannern und einem 9.4 T Scanner für experimentelle Tierversuche beherbergt. Ein drittes, vom MDC finanziertes Gebäude, das 2600 m² an Labor- und Bürofläche umfassen wird, wird im Frühjahr 2010 begonnen werden. Die Fertigstellung ist für 2012 geplant.

Ein großer Schritt in Richtung auf dieses Ziel ist mit der 2009 gelungenen Bestellung von Thoralf Niendorf getan worden. Er ist MDC-Gruppenleiter für die hochauflösende MR-Technik am ECRC und gleichzeitig W3-Professor an der Charité. Thoralf Niendorf ist ein führender Spezialist für bildgebende MR-Technik und hat Forschungserfahrung sowohl im industriellen als auch im akademischen Bereich.

Die geplante ECRC-Struktur wird von hoher Bedeutung sein für den Aufbau und die Arbeit mit der Helmholtz-Kohorte, die 200 000 Probanden und Patienten aus Deutschland, einschließlich 40 000 Personen aus der Berliner Region.

Das Berliner Institut für Medizinische Systembiologie (BIMSB) des MDC

Vor der biomedizinischen Forschung steht gegenwärtig die große Herausforderung, die Funktionsweise der einzelnen Komponenten biologischer Systeme (Gene, RNS, Proteine, usw.) in ihrem Zusammenwirken in komplexen dynamischen Netzwerken höherer Struktureinheiten (Zellen, Gewebe, Organismen) des Lebens zu verstehen.

Große Schritte im Erkenntnisgewinn werden oft durch ein multidisziplinäres Herangehen erreicht. Dies hat schon unser Namenspatron, Max Delbrück, bewiesen, der als Physiker in Zusammenarbeit mit dem Genetiker Timoffeéf Ressovsky die Molekularbiologie vor nahezu 50 Jahren begründet hat. Eine ganzheitliche Sicht ist auch für das Verständnis der bei Krankheitsprozessen ablaufenden Veränderungen essentiell. Mit den heute zur Verfügung stehenden neuen Technologien und Hochdurchsatzverfahren sind wir in der Lage, sehr große Datenmengen zu erzeugen, die nur mittels mathematischer Ansätze analysiert und verstanden

science." This location will permit stronger ties in the education of university and PhD students.

Training the "systems biologists" of the future is a challenge that needs to be addressed in a well thought-out way. Thus education is an important component of the BIMSB, and currently the most important element is the joint PhD program established between the MDC and New York University. This offers an original model whereby two institutes on different continents can strengthen their ties and work together in a practical way through sharing students, assigned to two labs, who split their time between them. Although the project formally started only this year, it has already produced a publication in *Nature Methods*, and other papers are in the pipeline.

The state of Berlin has declared its intention to provide the funds for the construction of the new building. In a new type of "integrative" lab, scientists using experimental approaches will team up with those using theoretical approaches. In order to optimally establish the many themes, disciplines, and technologies of this research area, 25 groups (including at least five W3 professorships) and five technology platforms are planned.

The BIMSB will also provide a basis for the development of the German Center for Cardiovascular Research (Deutsches Zentrum für Herz-Kreislaufforschung, DZHK; see below) through an emphasis on cardiovasular and metabolic diseases (particularly in the area of metabolomics).

German Center for Cardiovascular Research (Deutsches Zentrum für Herz-Kreislaufforschung, DZHK) and German Institute for Cardiovascular Research (Deutsches Institut für Herz-Kreislaufforschung, DIHK)

Cardiovascular diseases are the most common cause of death worldwide. They arise from a complex mixture of genetic and environmental factors – an enormous challenge as researchers try to track them back to specific genes, mechanistic processes in cells and intricate body systems. Such multifactorial diseases pose special problems including finding patients with a particular profile, extrapolating from a very small number of cases, and thoroughly integrating

werden können. Die immer stärkere Verschränkung verschiedener Wissenschaftsdisziplinen wie Biologie, Chemie, Physik, Mathematik, Ingenieurwissenschaften und auch Sozialwissenschaften ist eine wichtige und spannende Entwicklung. Diese Entwicklung muss sich langfristig auch in der Ausbildung junger Wissenschaftler niederschlagen.

In den vergangenen zwei Jahren hat das MDC ein Konzept erarbeitet, in dem diesen Entwicklungen Rechnung getragen wird. Das MDC plant mit der Errichtung des Berliner Institut für Medizinische Systembiologie (BIMSB) als Teil des MDC eine wichtige Ausweitung seiner Aktivitäten im Bereich Systembiologie. Im April 2009 wurde das wissenschaftliche Konzepte von einem internationalen Gremium weltweit anerkannter Experten aus den relevanten Disziplinen begutachtet. Details des Konzeptes und seiner gegenwärtigen Verwirklichung werden in diesem Bericht ebenfalls dargestellt. Hier beschränke ich mich auf einen kurzen Bericht über den Entwicklungsstand dieses Institutes.

Die Gründung des BIMSB wurde durch die Förderung des BMBF über die Initiative „Innovation und Spitzenforschung in den Neuen Ländern" und zusätzlicher Mittel des Senats von Berlin ermöglicht. Nikolaus Rajewsky hat diese Entwicklungen entscheidend vorangetrieben und übernahm die Funktion des Sprechers und Koordinators des Gesamtprojekts. Erste neue Forschungsgruppen konnten bereits eingerichtet werden, weitere werden folgen. Das MDC wird seine enge Zusammenarbeit mit den Berliner Universitäten und der Charité über das BIMSB vertiefen. Gemeinsam mit den Partnern wurde für das BIMSB ein Standort auf dem Nordcampus der Humboldt-Universität zu Berlin in der Nähe ihres Institutes für Theoretische Biologie, dem Campus Mitte der Charité und des Berliner „Bernstein Zentrums für Computational Neuroscience" gefunden. Dieser Standort ermöglicht auch eine stärkere Einbindung in die Ausbildung von Studenten und Doktoranden.

Die Ausbildung von "Systembiologen" ist ein wichtiges Anliegen. Ein besonderer Bestandteil des Ausbildungskonzeptes ist ein gemeinsames Doktoranden-Programm von BIMSB, MDC, und des Zentrums für Funktionelle Genomik, New York University. Dies ist ein neuartiges Modell einer interkontinentalen Zusammenarbeit bei der Ausbildung von Doktoranden, die ihre

approaches from bioinformatics/systems biology, clinical, and experimental approaches. Germany has a strong reputation in cardiovascular research. But there remain clear deficits that need to be addressed, particularly as cardiovascular diseases have a lower priority in the national research agenda than other health problems that pose a lesser burden on the health care system. Cardiovascular diseases must be approached in a highly interdisciplinary way, through a meaningful blend of basic and clinical research, and our efforts can be improved through coordination, networking and the common use of infrastructure.

Together with partners from medical faculties and the German Society for Cardiology, the MDC is spearheading an effort to substantially increase support for this research through the establishment of a German Center for Cardiovascular Research (Deutsches Zentrum für Herz-Kreislaufforschung, DZHK). It will consist of a German Institute for Cardiovascular Research (Deutsches Institut für Herz-Kreislaufforschung, DIHK) as an extension of the MDC and integrate excellent ongoing work across Germany by establishing new interconnected partner institutes. They will be chosen and their budgets will be established through a transparent, competitive review process. The DIHK will be set up by the MDC in close collaboration with the Charité. Besides its research activities, it will manage the administration of the partner institutes and provide technology platforms as well as other infrastructures. The DIHK will be able to draw on all the resources and infrastructure of the ECRC and BIMSB.

The overall mission of the DZHK can be summarized as following:

- To become the key national institution for basic and clinical cardiovascular research in Germany;
- To establish and provide national technological platforms for cardiovascular research;
- To establish a nationwide training platform for graduate and post-graduate;
- To inform the public about scientific and medical progress in this area;
- To intensify contacts with the pharmaceutical industry.

Arbeiten zwischen jeweils zwei Forschungsgruppen an beiden Standorten aufteilen. Obwohl das Projekt erst in diesem Jahr gestartet ist, konnten bereits eine Publikation in der Zeitschrift „Nature Methods“ und eine Reihe weiterer Manuskripte veröffentlicht werden.

Das Land Berlin hat seine Absicht bekundet, die Mittel für den Bau des neuen Gebäudes zur Verfügung zu stellen. Es wird sich durch neuartige „integrative Laboratorien“ mit einer starken Verzahnung experimenteller und theoretischer Arbeitsplätze auszeichnen. Das Gebäude wird Platz für bis zu 25 Forschergruppen (davon mindestens fünf W3-Professuren) und fünf Technologieplattformen bieten.

Das BIMSB wird darüber hinaus eine wichtige Unterstützung für den Aufbau eines Deutschen Zentrums für Herz-Kreislauf-Forschung (DZHK) bilden (siehe weiter unten), das sich auf die Erforschung kardiovaskulärer und metabolischer Erkrankungen konzentriert.

Deutsches Zentrum für Herz-Kreislaufforschung (DZHK) und Deutsches Institut für Herz-Kreislaufforschung (DIHK)

Erkrankungen des Herz-Kreislaufsystems sind die häufigste Todesursache weltweit. Für die Forschung stellen sie eine besondere Herausforderung dar, da zu ihnen eine Vielzahl genetischer und umweltbedingter Faktoren beitragen. Gerade in den letzten Jahren sind aber neue Ansätze (z.B. in der Systembiologie) entwickelt worden, die dem komplexen Charakter dieser Erkrankungen Rechnung tragen.

Auf dem Gebiet der kardiovaskulären Forschung leisten deutsche Arbeitsgruppen exzellente, international vielbeachtete Beiträge. Gleichwohl bleibt es eine wichtige Aufgabe, vorhandene Stärken besser zu nutzen und auszubauen. Insbesondere sind eine bessere Zusammenarbeit und Koordination bestehender Forschungsaktivitäten und der Aufbau neuer Forschungsgruppen und -infrastrukturen notwendig, um die Überführung von Ergebnissen aus der Grundlagenforschung in die Klinik zu fördern und die internationale Sichtbarkeit weiter zu steigern. Dabei müssen experimentelle und klinische Ansätze eng zusammengeführt werden. Darüber hinaus ist festzustellen, dass national der Erforschung kardiovaskulärer Erkrankungen bislang ein wesentlich geringerer Stellenwert zugemessen wurde

A detailed proposal for the DZHK has been submitted to the Federal Science Ministry. A call for applications as partner institutes in the DZHK is envisaged for 2010.

The Development of an In Vivo Pathophysiology Laboratory for long-term studies at the MDC

The success of the MDC is largely based on the generation and study of animal models – including traditional systems such as the fly, mouse, zebrafish, and *C. elegans*, important biomedical organisms such as the rat, and some more "exotic" animals including planaria and the naked mole rat (*Heterocephalus glaber*). Work on genetics and fundamental mechanisms within these systems is proceeding at a rapid pace, but extrapolating this work to human medicine requires extensive, creative, long-term phenotyping of animals. To study the impact of targeted changes in genes on the function of complex organ systems such as the cardiovascular or nervous systems, or the effectiveness of new therapies in animal models, state-of-the-art methods must be used to investigate living animals (*in vivo* technologies). This has two main goals: to acquire new basic knowledge and to develop and test new treatments.

Since our facilities for this type of work are currently rather limited, we have developed a major proposal for the construction of an *In Vivo* Pathophysiology Laboratory (IPL) which has received an enthusiastic review and is considered essential by the Scientific Advisory Board, external reviewers and within the Helmholtz Association.

The IPL will accommodate research laboratories that are implementing and further developing cutting-edge phenotyping tools (e.g., imaging and the *in-vivo* recording of cellular activity). Additionally, the facility will provide a large laboratory devoted to behavioral and physiological studies.

The IPL will be an invaluable infrastructure that supports all relevant MDC groups and projects such as the DIHK. We foresee very fruitful collaborations between the screening unit at the FMP and the imaging facilities in continuing our studies of the effects of probes, inhibitors, and drug candidates on organisms

als anderen Erkrankungen mit einer geringeren Belastung des Gesundheitssystems.

In Zusammenarbeit mit den medizinischen Fakultäten verschiedener Universitäten und der Deutschen Gesellschaft für Kardiologie bemüht sich das MDC um einen Ausbau und eine stärkere Vernetzung der Forschung durch die Einrichtung eines Deutschen Zentrums für Herz-Kreislaufforschung (DZHK). Dieses wird aus einem Deutschen Institut für Herz-Kreislaufforschung (DIHK) als Teil des MDC und mehreren Partnerinstitutionen in ganz Deutschland bestehen.

Die Partnerinstitute werden in einem transparenten Wettbewerb ausgewählt. Das MDC wird das DIHK in enger Abstimmung mit der Charité gründen. Neben seinen Forschungsaufgaben wird es Plattformtechnologien und andere Infrastrukturen zur Verfügung stellen. Das DIHK wird ebenfalls die Ressourcen von ECRC und BIMSB nutzen können.

Die Mission des DZHK kann wie folgt zusammengefasst werden:

- *Es soll die nationale Leitinstitution für die kardiovaskuläre Grundlagen- und klinische Forschung in Deutschland werden.*
- *Es soll national nutzbare technologische Plattformen für die kardiovaskuläre Forschung bereithalten.*
- *Es soll eine deutschlandweit nutzbare Plattform für die Ausbildung von Graduierten und Postgraduierten werden.*
- *Es soll die Öffentlichkeit über den wissenschaftlichen Fortschritt und seine medizinische Anwendung informieren.*
- *Es soll auf seinem Wirkungsfeld den Kontakt zur pharmazeutischen Industrie intensivieren.*

Ein entsprechender detaillierter Vorschlag für die Errichtung des DZHK wurde beim Bundesministerium für Forschung eingereicht. Die Einladung, Partnerinstitut im DZHK zu werden, wird an alle einschlägigen Institutionen im Jahr 2010 ergehen.

Errichtung eines In-vivo *Pathophysiologielabors*

Der bisherige Erfolg des MDC beruht zum großen Teil auf die Erzeugung und Analyse von Modellorganis-

as a whole. The IPL will permit us to combine mechanistic studies of the cellular activity of these molecules and the effects that they have on model organisms.

Although the project has been very positively reviewed within the framework of Project-oriented funding (POF) applications, the main mechanism for allocating funds within the Helmholtz Association, there are still some hurdles to pass, before it becomes a reality. We are hopeful that it will be included in the priorities in health research activities to be determined by the Helmholtz Senate in early 2010.

We regard the establishment of the IPL as essential to the future of the MDC. A deeper phenotyping of existing and new mouse models as well as other organisms is a logical and essential next step toward understanding human diseases.

Attracting excellent scientists and public awarness of the MDC: two sides of a coin

Under the leadership of Walter Birchmeier, the MDC has been very successful in recruiting excellent new junior and senior faculty, several of whom were recognized with major awards during the reporting period.

Jana Wolf, of the HU Berlin, established a research group in early 2008 within the framework of the Helmholtz Alliance in "Systems Biology" and has received additional funding through the BMBF FORSYS Program.

Another recently recruited junior group leader, Dr. Francesca Spagnoli, an MD/PhD, was awarded a one-million Euro grant from the European Research Council (ERC). Her group was one of 240 labs throughout Europe chosen by the ERC for the award. Before she came to the MDC, Francesca Spagnioli was a postdoctoral fellow at Rockefeller University, performing very successfully research on stem cells. At the MDC, she hopes to learn to transform liver cells into pancreatic cells, such as the insulin-producing beta cells, which could have major implications for the treatment diabetes mellitus.

In 2008 the biochemist and neurobiologist Dr. Jan-Erik Siemens was one of eight junior researchers to the annual Sofja Kovalevskaja Award of the Alexander

men. Das gegenwärtige Repertoire schließt traditionell genutzte Spezies, wie Taufliege, Maus, Zebrafisch und C. elegans *ebenso ein wie die Ratte als wichtiges Objekt für biomedizinische Studien sowie einige eher „exotische" Tiere wie* Planaria *oder den Nacktmull (*Heterocephalus glaber*). Um die Auswirkungen einer gezielten Veränderung eines Gens auf die Funktion komplexer Organsysteme wie das Herz-Kreislaufsystem oder das Nervensystem zu untersuchen oder die Wirksamkeit neuer Therapien im Tiermodell zu testen, sind verschiedene ‚state of the art' Messverfahren am lebenden Tier (‚*in vivo*-Technologien') unabdingbar. Die umfangreiche Analyse von Tiermodellen ist doppelt wichtig: für neue grundlegende Erkenntnisse und zur Entwicklung und Überprüfung neuer Behandlungskonzepte.*

Da unsere verfügbaren Ressourcen für diese Art von Untersuchungen noch immer sehr begrenzt sind, haben wir uns entschlossen, einen Antrag zur Errichtung eines In-vivo *Pathophysiologielabors (IPL) zu stellen. Die Notwendigkeit, ein solches Labor zu errichten, wurde durch externe internationale Gutachten und von den Aufsichtsgremien des MDC, dem Kuratorium und seinem Wissenschaftlichen Ausschuss, ausdrücklich bestätigt.*

Das IPL soll mit modernsten Technologien für eine umfassende Analyse des Verhaltens, der Physiologie und der Pathophysiologie von Labortieren (Nagern) ausgestattet werden, wie z.B. bildgebende Verfahren oder in-vivo-*Messungen zellulärer Aktivitätsmuster. Die Weiterentwicklung bestehender und die Entwicklung neuer Methoden anhand unterschiedlicher Fragestellungen werden ebenfalls im Mittelpunkt der geplanten Aktivitäten stehen. Das IPL wird eine wichtige Infrastruktur für die Grundlagenforschung, die klinische Forschung und insbesondere für das DIHK darstellen.*

In einer sehr fruchtbaren Kooperation zwischen MDC und FMP werden in der Screening-Unit am FMP neue bioaktive Substanzen identifiziert. Im IPL sollen diese, z.B. mit Hilfe bildgebender Analyseverfahren, auf ihre Wirksamkeit im Tier hin analysiert werden.

Trotz der positiven Begutachtung im Rahmen der Programmorientierten Forschung (POF) der Helmholtz-Gemeinschaft, der Hauptquelle der Finanzierung in der Helmholtz-Gemeinschaft, sind einige Hürden zu über-

von Humboldt Foundation (AvH). The award stipend has enabled him to return to Germany and establish a research group at the MDC in May, 2009, after four years as postdoctoral fellow at the University of California, San Francisco, USA.

The MDC is financing two junior groups, those of Björn Christian Schroeder (previously of the University of California, San Francisco) and James Poulet (École Polytechnique Fédérale de Lausanne, Switzerland), within the excellence cluster "NeuroCure: Towards a better outcome of neurological disorders." The groups started at the MDC in 2009. Schroeder is working on the Berlin-Buch campus and Poulet is at the Charité campus in Berlin-Mitte until a new building is constructed for NeuroCure in the city center.

Markus Landthaler, of Rockefeller University in New York, is the first junior group leader to be recruited within the framework of the BIMSB.

The MDC continues to recruit scientists for our ongoing projects and new activities. Major ongoing recruitments include filling professorships in the areas of Epidemiology, Metabolism, Cardiovascular research, and Systems Biology.

The recruitment of excellent PhD students is also a priority for the institute and is based on international calls and a rigorous selection procedure. The programs offered to PhD students continue to expand and now include lectures, courses, seminars, research visits to other laboratories, etc. The MDC is now home to several graduate schools that have been established through grants with the Helmholtz Association and have received a total of 7.2 Mio. g funding to run over the next six years. These include the graduate school "Molecular Cell Biology," which comprises the International PhD programme in Molecular Cell Biology, the International Helmholtz Research Schools in Molecular Neurobiology (MolNeuro) and Translational Cardiovascular and Metabolic Medicine (TransCard), and the MDC-NYU Exchange Program in Systems Biology. Each of these projects springs from the initiative of one or more of our research labs and aims to enhance our current offerings for PhD students. At the same time, we intend to better integrate these programs through a core PhD course held in the

winden, bevor das Vorhaben Realität werden kann. Wir hoffen sehr, dass es als prioritäre Maßnahme aufgenommen wird, die im Frühjahr 2010 vom Helmholtz-Senat festgelegt werden.

Nach unserer Auffassung ist die Errichtung des IPL essentiell für die Zukunft des MDC. Eine eingehende phänotypische Charakterisierung existierender und neu zu entwickelnder Tiermodelle ist ein grundlegender und unverzichtbarer Schritt für das bessere Verständnis menschlicher Krankheiten.

Einwerbung von exzellenten jungen Wissenschaftlern und die öffentliche Wahrnehmung des MDC sind zwei Seiten einer Medaille

Unter der Leitung von Walter Birchmeier hat das MDC neue hochtalentierte junge Wissenschaftler und exzellente erfahrene Professoren gewinnen können. In den Jahren 2008 und 2009 haben sieben neue Nachwuchsgruppen ihre Arbeit am MDC aufgenommen.

Jana Wolf, Humboldt-Universität zu Berlin, hat ihre Forschungsgruppe im Rahmen der Helmholtz-Allianz „Systembiologie" und durch zusätzliche Mittel über das FORSYS-Programm des BMBF im Frühjahr 2008 am MDC etabliert.

Francesca M. Spagnoli, Rockefeller University, New York und Matthew Poy, ETH-Zürich, haben ebenfalls in 2008 ihre Arbeit am MDC aufgenommen. Im September 2009 erhielt Francesca Spagnoli vom Europäischen Forschungsrat (engl. Abkürzung ERC) einen von 240 vergebenen ERC starting grants von über 2500 Bewerbern.

Jan Erik Siemens, University of California, San Francisco, der im Sommer 2008 den Sofia-Kovalevskaja-Preis der Alexander von Humboldt Stiftung erhielt, hat seine Arbeit im Mai 2009 am MDC aufgenommen.

Im Exzellenzcluster „NeuroCure: Towards a better outcome of neurological disorders" finanziert das MDC zwei Nachwuchsgruppen. Björn Christian Schroeder, University of California, San Francisco, und James Poulet, Ecole Polytechnique Fédérale de Lausanne, Schweiz, haben in 2009 ihre Arbeit am MDC gegonnen. Björn Schroeder arbeitet am MDC in Berlin-Buch und James Poulet an der Charité, Campus Berlin-Mitte, bis das neue NeuroCure Gebäude in Berlin-Mitte bezogen werden kann.

fall; the 2009 session lasted two weeks and comprised talks by the heads of core facilities and investigators from our research programs. Additionally, retreats from students of the MDC and FMP were held in 2008 and 2009, organized by the students and the PhD programs.

The MDC is mainly financed by public resources. It is therefore highly motivated to raise awareness of the institute among non-scientists, who will eventually be affected by our work and the applications it produces. In additions, these efforts are essential in giving young people a first-hand look at science and, hopefully, stimulating many of them to choose a scientific career. A major activity is the Gläsernes Labor, operated by the BBB Management GmbH on the Berlin-Buch campus with significant support from the MDC. It continues to welcome huge numbers of visitors, particularly school children, for hands-on experiments in the lab and an encounter with modern biology. In 2008 the teaching labs were fully booked, receiving over 10,000 pupils and their teachers.

Our very active press office disseminates news about our findings to the media and coordinates our interactions with the public in that regard. The publication of *Geneticists in Berlin-Buch*, a collection of articles about three famous scientists associated with the campus, explores our rich history. Finally, we are bringing out other unique publications to give the research community and the wider public insights into work and life on our campus. 2008 saw the publication of the book *Translations: From today's science to tomorrow's medicine in Berlin-Buch*, a large, coffee-table book with lavish photographs and a wide range of easy-to-understand stories from our current work.

A last remark

To conclude, I would like to emphasize that it has been a pleasure – and a great challenge – to assume the directorship of a thriving, growing. "Molecular medicine" and "translational research" are buzzwords that can be heard across the world. As outlined above, at the MDC we are pursuing these themes in a very dynamic, flexible and concrete way. As we make the transition from the current size and scope of our

Markus Landthaler, Rockefeller University, New York, ist der erste Nachwuchsgruppenleiter, der für das BIMSB gewonnen werden konnte.

Die Auswahl exzellenter Doktorstudenten erfolgt am MDC über ein strenges Auswahlverfahren nach internationaler Ausschreibung. Unser PhD-Programm bietet den Doktoranden mehrere Vorlesungsreihen, Kurse und Seminare, Kongressteilnahmen, Forschungsaufenthalte in anderen Laboren und vieles mehr. Ende 2009 erfolgte bereits die achte Ausschreibung unseres PhD-Programms, das wir gemeinsam mit der Humboldt-Universität zu Berlin in 2001 gestartet haben. Das MDC hat für alle Doktoranden, die ab Juli 2006 mit ihrer Arbeit am MDC begonnen haben, ein einheitliches Curriculum etabliert. Mit zentralen Mitteln der Helmholtz-Gemeinschaft, die das MDC in den letzten zwei Jahren für drei Programme einwerben konnte, stehen nun insgesamt 7,2 Mio. € über eine Laufzeit von insgesamt 6 Jahren zusätzlich für eine strukturierte Ausbildung zur Verfügung. Zu diesen Programmen gehören die Helmholtz-Graduiertenschule „Molecular Cell Biology", das Helmholtz-Kolleg „Molecular Neurobiology" und das Helmholtz-Kolleg "Translational Cardiovascular and Metabolic Medicine – TransCard". Im Gegensatz zu den Kollegs, die thematisch fokussiert sind, ist die Graduiertenschule als disziplinübergreifende Dachstruktur angelegt. Wie in den Jahren zuvor wurden auch in 2008 und 2009 PhD-Retreats gemeinsam von Doktoranden des MDC und FMP organisiert.

Das MDC wird vorwiegend mit öffentlichen Mitteln finanziert. Wir sind deshalb sehr daran interessiert, der Öffentlichkeit die Ergebnisse unserer Arbeit näher zu bringen. Wir hoffen auf diesem Weg, jungen Menschen einen Einblick in die Wissenschaft aus erster Hand zu bieten und sie zu einer wissenschaftlichen Laufbahn zu ermutigen.

Eine wichtige Aktivität ist hierbei die Unterstützung des Gläsernen Labors, das auf dem Bucher Campus von der BBB Management GmbH betrieben wird. Es wird vor allem von Schulkindern besucht, die mit ersten eigenen molekularbiologischen Experimenten einen Einblick in die moderne Biologie erhalten. 2008 und 2009 war das angebotene Programm durch ca. 10.000 Schülerinnen und Schüler und ihren Lehrkräften vollständig ausgebucht.

organization to a larger, multi-faceted one, we are taking care to tighten our interactions with our partners, on the regional, national and international levels, through activities such as the BIMSB and DIHK. These steps are necessary to achieve the primary goals of our campus, to integrate new developments, and to preserve a multifaceted range of research activities (and here, we regard the establishment of the IPL as a necessity).

These developments are only possible because we have attained a critical mass that stimulates further growth. And our efforts can only come to fruition in a culture which has a critical mass of superlative basic science. By building on existing strengths, we are moving in a clearly defined direction that promises to have a major impact on the field of human health. Finally, excellent science requires excellent scientists. Thus increasing the attractiveness of the MDC to the best scientists around the world is central to all the measures we are planning.

*Unsere Stabstelle für Presse- und Öffentlichkeitsarbeit verfasst zahlreiche Berichte für die Medien und koordiniert unsere Interaktionen mit der Öffentlichkeit. Mit der Veröffentlichung des Buches „*Genetiker in Berlin-Buch*", eine Sammlung von Artikeln zu drei berühmten Wissenschaftlern, die früher auf dem Campus gearbeitet haben, tragen wir unserer vielschichtigen Vergangenheit Rechnung. Für die Abbildung der Gegenwart haben wir in 2008 das Buch: "*Translations: From today's science to tomorrow's medicine in Berlin-Buch" *veröffentlicht. Es ist eine umfangreiche, unterhaltende Geschichten- und Anekdotensammlung mit allgemein verständlichen Darstellungen und eindrucksvollen Fotos unserer Wissenschaftler und deren Forschung.*

Schlusswort

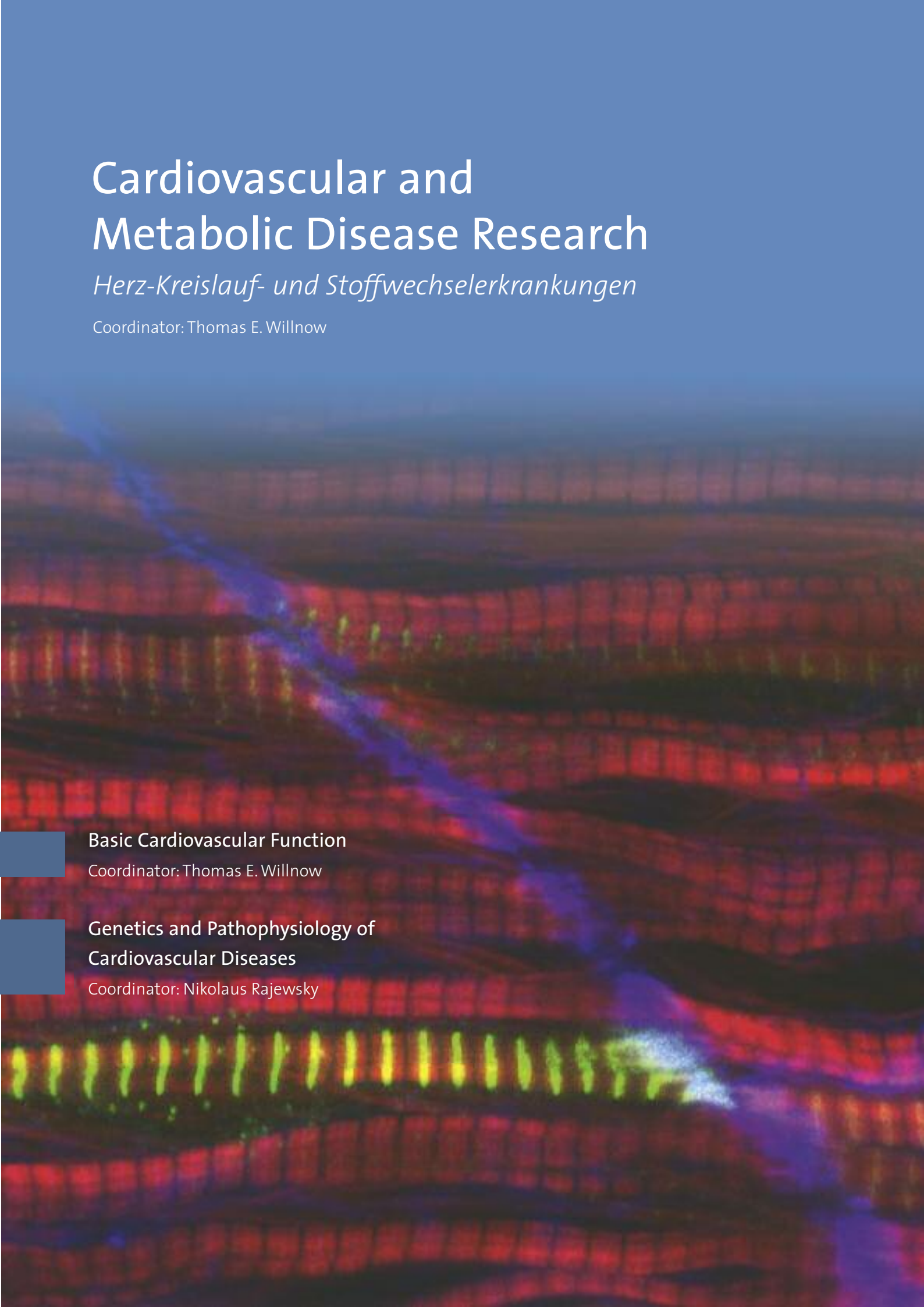
Es ist für mich eine große Freude und eine sehr anspruchsvolle Aufgabe, die Leitung einer so erfolgreichen und dynamischen wissenschaftlichen Institution, wie sie das MDC darstellt, übernommen zu haben. Das MDC hat die Herausforderung, die mit den Schlagworten „Molekulare Medizin" und „Translationsforschung" verbunden sind, seit seiner Gründung angenommen und mit großem Enthusiasmus verfolgt. Dabei hat sich eine dynamische Institutsstruktur herausgebildet, die – bei Beibehaltung des Grundkonzepts – neue Entwicklungen integrieren kann. Dies ist eine wichtige Voraussetzung für die aktuelle Herangehensweise: unter Beibehaltung einer facettenreichen Forschungseinheit (dazu ist der Neubau des IPL erforderlich!) die regionale, nationale und internationale Verflechtung, u.a. durch den Aufbau von BIMSB und DIHK, voranzutreiben.

Eine entscheidende Voraussetzung für die Verwirklichung der hier dargestellten Konzepte ist dadurch gegeben, dass das MDC eine kritische Masse an exzellenter Forschung erreicht hat. Wir sind davon überzeugt, dass wir aufbauend auf unseren Stärken hervorragende Ergebnisse in der Gesundheitsforschung erreichen werden. Vor allem aber muss es das Ziel des MDC bleiben, im weltweiten Wettbewerb die besten Wissenschaftler für sich zu gewinnen. Denn: Exzellente Forschung gedeiht dort, wo sich exzellente Forscher treffen und ihre Ideen gemeinsam umsetzen.

Cardiovascular and Metabolic Disease Research

Herz-Kreislauf- und Stoffwechselerkrankungen

Coordinator: Thomas E. Willnow



Basic Cardiovascular Function

Coordinator: Thomas E. Willnow

Genetics and Pathophysiology of Cardiovascular Diseases

Coordinator: Nikolaus Rajewsky

Cardiovascular and Metabolic Disease Research

Herz-Kreislauf-Krankheiten und metabolisches Syndrom

Thomas E. Willnow

Cardiovascular disease is the most common cause of death worldwide. In industrialized nations, this state of affairs has been the case since early in the 20th century. In the 21st century, cardiovascular disease has become global. For instance, almost half the disease burden in low-and-middle-income countries of Europe and Central Asia now comes from cardiovascular diseases. In the United States, 60 million people have known cardiovascular disease, 50 million have hypertension, 13 million have coronary disease, and 5 million have had a stroke. These figures comprise 1 in 5 of the US population. Figures for the European Union are no different. Five percent of the US and EU population are known to have type 2 diabetes mellitus. This "adult-onset" diabetes is now the most common cause of diabetes in children. Kidney disease leading to end-stage renal failure is increasing exponentially and diabetes, coupled with hypertension, is the most common cause. The number of people with a body mass index (BMI) >30 is approaching 30% of the population and half the population is overweight (BMI >25%). These figures are alarming, since it could mean a reversal of the trend throughout the last century of an ever-increasing life expectancy in our societies.

Learning to prevent and treat cardiovascular and metabolic diseases will require understanding the underlying genetic and pathophysiological mechanisms, and this approach offers a passport to *in vivo* translational approaches. Thus research in this program aims at elucidating the genetic pathways which regulate the normal function of the cardiovascular system and metabolism and whose defects cause human diseases. Ultimately, the identification of such disease genes will lead to a better understanding of pathological processes, to improved diagnoses, and to new concepts in therapy.

Toward these goals, we use functional genomics approaches to study disease processes in model systems ranging from the zebrafish, to the mouse and rat, and we compare our findings to studies conducted in humans (and vice versa). The studies are performed by group leaders at the MDC in close collaboration with clinicians at the Charité, University Medicine of Berlin.

Weltweit sind Erkrankungen des Herz-Kreislauf-Systems inzwischen die häufigste Todesursache. Galt dies seit Beginn des 20. Jahrhunderts zunächst nur für die Industrienationen, trifft dieser Befund im 21. Jahrhundert nunmehr global zu. So ist die Hälfte der Erkrankungen in den europäischen und zentralasiatischen Gesellschaften mit geringem oder mittlerem Nationaleinkommen heute diesem Komplex von Krankheiten zuzurechnen.

In den USA leiden 60 Millionen Menschen an Herz-Kreislauf-Krankheiten. 50 Millionen haben Bluthochdruck, 13 Millionen leiden an Herzkrankheiten, und 5 Millionen hatten einen Schlaganfall. Damit ist also jeweils einer von fünf Einwohnern der USA von einer Erkrankung des Herz-Kreislaufsystems betroffen. Hinzu kommt, dass 5 Prozent der US-Bürger an Diabetes vom Typ 2 leiden.

Auch in der Europäischen Union verhält es sich nicht anders. Fünf Prozent der EU-Bürger leiden ebenfalls an Diabetes vom Typ 2. Diese typischerweise erst im Erwachsenenalter einsetzende Form der Zuckerkrankheit ist heute bereits die häufigste Form von Diabetes im Kindesalter. Als Folge nehmen Nierenerkrankungen bis hin zum Nierenversagen exponentiell zu.

Eine große Rolle spielt in diesem Zusammenhang auch das Übergewicht. 30 Prozent der Bevölkerung erreichen den gefährlichen BMI-Index-Wert (body mass index) von über 30. Bereits 50 Prozent der Bevölkerung haben einen Index von mehr als 25, was bereits Übergewicht anzeigt. Das sind alarmierende Befunde. Sie könnten den Trend ständig steigender Lebenserwartung umkehren, der das vergangene Jahrhundert gekennzeichnet hat.

Um Erkrankungen des Herz-Kreislauf-Systems verhüten oder erfolgreich behandeln zu können, muss man die zu Grunde liegenden genetischen und pathophysiologischen Mechanismen aufklären. Ziel unseres Forschungsprogramms ist es deshalb, genetische Netzwerke aufzuklären, welche die normale Funktion des kardiovaskulären Systems regulieren und die, wenn sie defekt sind, zu Erkrankungen führen. Letztlich wird die Aufklärung solcher krankheitsverursachenden Gene zu einem besseren Verständnis pathologischer Prozesse, aber auch zu besseren diagnostischen Verfahren und schließlich zu neuen Therapiekonzepten führen.

Im Hinblick auf diese Zielsetzung betreiben wir funktionelle Genomforschung in Modellsystemen wie Zebrafisch, Maus oder Ratte. Wir vergleichen die gewonnenen Befunde mit solchen, die am Menschen erhoben werden konn-

Our research activities are organized in two topics, (i) Basic Concepts of Cardiovascular Function and (ii) the Genetics and Pathophysiology of Cardiovascular Disease. Both topics center around the comparative analysis of model systems and human diseases. Close interactions between scientists in these two topics promote synergies between projects using forward and reverse genetics. They stimulate exchange between projects in basic science and disease-oriented research and promote the transfer of new ideas and concepts from the "bench" to the "bedside". Interestingly, many of the mechanisms that contribute to cardiovascular diseases also play a fundamental role in other body systems, such as the nervous system and the brain, so scientists from the program have made important contributions to the MDC's other main research topics.

Topic 1: Basic concepts of Cardiovascular Function

The aim is to characterize major *regulatory pathways* that underlie normal cardiovascular function and to investigate these in cell culture and organ systems. As well as studying the adult organism, an emphasis is placed on *developmental pathways*, as they represent paradigms for differentiation processes in normal and diseased adult tissues. Major focuses of our research activities in this topic are cardiovascular signal transduction and cell biology as well as development and regeneration of the cardiovasculature.

Some recent highlights from the program include outstanding work by some of our younger group leaders. For example, in 2008 Michael Gotthardt's lab discovered that the Coxsackie-adenovirus receptor (CAR), primarily known for its role in allowing the virus to infect cells, also plays a crucial role in heart development and function. CAR is a tight junction protein and ties cardiac cells together to help transmit electrical signals between them. Defects in other cell-contact proteins have been linked to arrhythmia, but so far CAR had not been implicated. Using conditional knockout mice, the lab discovered that a loss of CAR disturbs the localization of ion channels called connexins, which apparently must be clustered in the cell membrane to coordinate the transmission of

ten. Dieser wissenschaftliche Ansatz erfordert die enge Zusammenarbeit grundlagen-orientierter Arbeitsgruppen des MDC mit klinischen Gruppen in der Charité – Universitätsmedizin Berlin.

Unser Forschungsprogramm gliedert sich in zwei Teilgebiete: 1) Grundlagen der Funktion des kardiovaskulären Systems und 2) Genetik und Pathophysiologie kardiovaskulärer Krankheiten. Beide Teilgebiete konzentrieren sich auf die vergleichende Analyse von Modellsystemen und Krankheiten des Menschen. Die enge Zusammenarbeit entlang dieser Forschungsstrategien eröffnet Synergien zwischen solchen Projekten, die vom Gen auf das Merkmal zielen und denen, die vom (Krankheits-)Merkmal ausgehend die genetischen Ursachen suchen. So ergibt sich ein logisches Ineinandergreifen von Grundlagenstudien und klinisch orientierter Forschung, was den Transfer von neuen Erkenntnissen vom „Labor" zum „Krankenbett" in einer sinnvollen Weise befördert.

Teilthema 1: Grundlagen der Funktion des Herz-Kreislaufsystems

Hier ist es das Ziel, die wichtigsten Regelkreise*, die der Funktion des Herz-Kreislauf-System zugrunde liegen, aufzuklären. Sie sollen sowohl in der Zellkultur als auch in intakten Organsystemen untersucht werden. Neben dem Studium des adulten Organismus werden auch* Regulationsnetzwerke der embryonalen Entwicklung *betrachtet, da sie modellhaft für Differenzierungsprozesse in pathologisch veränderten Geweben des Erwachsenen sind. Der Schwerpunkt der Untersuchungen auf diesem Gebiet liegt sowohl auf der Signaltransduktion in der Zelle als auch auf den Entwicklungs- und Regenerationsprozessen des kardiovaskulären Systems.*

Herausragende Ergebnisse im Rahmen dieses Programms haben insbesondere jüngere Forschergruppen erbracht. Hier ist Michael Gotthards Gruppe zu nennen, die herausfand, dass der Rezeptor des Coxsackie-Adenovirus (CAR) ein wichtiger Faktor in der Entwicklung und Funktion des kardiovaskulären Systems ist. CAR ist ein „tight junction protein", das Herzzellen miteinander verbindet und diese zur Übertragung elektrischer Signale befähigt. Defekte in anderen Zellkontakt-Proteinen wurden bereits mit Herz-Arrhythmie in Verbindung gebracht; CAR gehörte jedoch bislang nicht zu diesen Kandidatenproteinen. Mit Hilfe des sogenannten „conditional knock-out" in der Maus hatte die

electrical signals. More recent work by the lab has provided insights into how infections by the coxsackie virus cause heart damage. The work emphasizes CAR's significance as a potential target for therapies, while underlining the importance of understanding its functions in the healthy heart.

Salim Seyfried's group has been working on elucidating the mechanisms that control cardiac cell behaviors during early heart formation. Initially most cardiac cells are arranged within a flat sheet called the heart cone which undergoes a complex morphogenetic transformation to create the heart tube. The role of cell migrations or epithelial bending in this process has been unclear. Recent projects by the lab have shown how the differential expression of proteins in the left and right sides of the heart field lead to asymmetries that are crucial in giving the organ its proper shape. Time-lapse microscopy studies combined with the analysis of genetically modified zebrafish showed that cells migrate asymmetrically, which plays a central role in establishing different dorsoventral regions of the heart tube. Another finding of the group was that cardiac cells initially acquire their shapes independently of the function of the early heart. This finding revealed new connections between the way cardiac cells acquire shapes and contribute to the hearts' structure as a whole.

Topic 2: Genetics and Pathophysiology of Cardiovascular Diseases

Within this topic we aim to identify major genetic, genomic and pathophysiological mechanisms in patients and in animal models of cardiovascular disease. Based on our findings, novel concepts for improved diagnosis and therapeutic intervention are explored.

Over the past two years, several projects within the program have established new connections between genes and susceptibility to heart diseases and fundamental mechanisms that play a role in their development. A collaboration between the groups of Norbert Hübner, Friedrich Luft and clinician Jan Monti of Charité/HELIOS has shown that the gene Ephx2 contributes to heart failure and the arrhythmic beating that signals sudden cardiac death. The researchers

Gruppe zuvor feststellen können, dass die Ausschaltung des CAR-Gens die Lokalisierung bestimmter Proteine, den Connexinen, verändert. Connexine müssen offensichtlich als Cluster in der Zellmembran vorliegen, um die Übertragung elektrischer Signale zu koordinieren. Neuere Ergebnisse lieferten Einsichten in die von Coxsackie verursachte Herzschädigung. Sie betonen die Bedeutung von CAR als möglichem Ansatzpunkt für therapeutische Eingriffe, aber auch für das Verständnis der Funktion des gesunden Herzens.

Salim Seyfrieds Arbeitsgruppe untersuchte den Kontrollmechanismus für das Verhalten von Herzzellen während der embryonalen Organbildung. Am Beginn des Prozesses sind die Herzzellen als flache Schicht angeordnet (heart cone), aus der durch komplexe morphologische Veränderungen die Herzröhre entsteht. Wie dabei Wanderung und Richtungsänderung von Epithelien zustande kommen, war bislang unklar. Die Gruppe konnte zeigen, dass durch genau abgestimmte Expression von Proteinen auf der rechten und der linken Seite des Herzfeldes eine Asymmetrie entsteht, die entscheidend für die präzise Gestaltbildung des Organs ist. Mikroskopische Zeitverlaufsstudien an genetisch veränderten Zebrafischen zeigten den asymmetrischen Wanderungsverlauf der Zellen, der eine zentrale Rolle in der Ausbildung der dorsoventralen Anordnung der Herzröhre spielt. Darüber hinaus fand die Arbeitsgruppe heraus, dass Herzzellen ihre ursprüngliche Gestalt unabhängig von der Funktion der ersten Herzanlage annehmen. Dieser Befund zeigt neue Wege für das Verständnis der Gestaltbildung der Herzzellen und ihres Beitrags zur Gestaltbildung des ganzen Organs auf.

Teilthema 2: Genetik und Pathophysiologie von Herz-Kreislauf-Erkrankungen

Ziel ist hier, die genetischen und pathophysiologischen Mechanismen von kardiovaskulären Erkrankungen am Menschen und in Modellorganismen zu identifizieren. Unsere Befunde dienen der konzeptionellen Vorbereitung neuer Diagnose- und Therapieverfahren.

In den vergangenen zwei Jahren haben mehrere Forschungsprojekte des Programms neue Zusammenhänge zwischen Genvarianten, die zu Herzerkrankungen disponieren, und grundlegenden Mechanismen der embryonalen Herzentwicklung gefunden. Die Forschergruppen von Norbert Hübner, Friedrich Luft und dem Kliniker Jan Monti (Charité/HELIOS) konnten in einer Gemeinschaftsarbeit

combined computation and laboratory experiments in a study of a rat model for spontaneous hypertensive heart failure (SHHF). By crossing SHHF rats with another strain that is hypertensive but does not develop heart failure, they obtained a population of rats with various degrees of heart failure while all animals had high blood pressure. This allowed the scientists to peel apart factors that contribute to high blood pressure and heart failure. Ephx2 modifies a hormone called EET in cardiac muscle, which plays a role in coordinating heart cell contraction, relaxes blood vessels and helps protect the organ after some types of heart attacks. Extending the work to human patients recovering from heart failure, they discovered low levels of Ephx2 in patient tissue. An improperly functioning version of Ephx2 found in some people may lead to more severe damage in the aftermath of heart failure.

Another very active area within the program explores fundamental mechanisms by which cells regulate gene expression and the connections between these systems and heart functions and disease. One focus of the labs of Nikolaus Rajewsky and Matthias Selbach is the way microRNAs regulate hundreds of genes. Combining a method called SILAC (which labels amino acids with a stable, non-radioactive isotope) with mass spectrometry, the researchers obtained the first quantitative measurements of global changes in protein production in cells that express particular microRNAs. Sometimes these small molecules cause the destruction of messenger RNAs; in other cases they prevent the translation of mRNAs into proteins. The scientists found that single microRNAs use both methods as a sort of global "volume control" for the output of hundreds of genes, allowing the cell to tune protein production up and down. Prior to the development of this method, it was difficult to measure the amounts of molecules synthesized by cells under various conditions. In many cases, diseases are thought to arise from a change in the dosage of a particular protein found in certain types of cells, rather than its absolute presence or absence. The work provides a new method to get a handle on this issue.

nachweisen, dass das Gen Ephx2 eine wichtige Rolle bei der Entstehung der Herzinsuffizienz und der Arrhythmie spielt. Letztere ist ein Vorbote des plötzlichen Herztodes. Die Forscher kombinierten Computermodellstudien und Experimente an einem Rattenmodell für spontane hypertensive Herzinsuffizienz (SHHF). Sie kreuzten SHHF-Ratten mit einem anderen Stamm, der zwar Bluthochdruck, aber keine Herzinsuffizienz entwickelt. Sie erhielten so eine Population von Ratten mit abgestuften Ausprägungen von Herzinsuffizienz bei durchgehend bestehendem Bluthochdruck. Die Analyse ergab damit verschiedene Ursachen für Bluthochdruck und für Herzinsuffizienz. Im Herzmuskel wird die Wirkung eines Hormons mit der Bezeichnung EET durch Ephx2 verändert. Dieses Hormon ist wichtig für die Koordination von Herzmuskelkontraktion und Blutgefäßerweiterung und bildet einen Schutzfaktor beim Herzinfarkt. Bei der Ausweitung dieser Studien auf Herzpatienten, die sich in der Erholungsphase nach einer akuten Herzinsuffizienz befanden, wurden erniedrigte Ephx2-Werte im Patientengewebe beobachtet. Eine Fehlfunktion des Ephx2 könnte also bei manchen Patienten die Ursache für schwere Schädigungen in der Erholungsphase nach einer Herzinsuffizienz sein.

Ein weiteres sehr intensiv bearbeitetes Teilgebiet des Programms sind die fundamentalen Mechanismen der Regulation der Genexpression und ihrer Verbindung zu Herzfunktion und Herzversagen. Ein Schwerpunkt der Studien in den Laboratorien von Nikolaus Rajewsky und Matthias Selbach ist die Regulation der Expression von Hunderten von Genen durch microRNAs. Durch den koordinierten Einsatz der SILAC-Methode (bei der gewisse Aminosäuren durch stabile, nicht-radioaktive Isotopen markiert werden) und anschließender Massenspektrometrie konnten die Forscher erstmals quantitative Messungen von Veränderungen der Gesamtproduktion von Proteinen in Zellen erhalten, die microRNS exprimieren. In einigen Fällen bewirken diese kleinen Moleküle die Zerstörung von Boten-RNAs; in anderen Fällen hemmen sie die Translation der mRNA und damit die Entstehung der durch sie kodierten Proteine. Es zeigte sich, dass manche microRNS beide Regulationsmechanismen nutzen, um eine Art „Gesamtkontrolle" über die Ausbildung Hunderter verschiedener Proteine auszuüben. Solche wissenschaftlichen Untersuchungen waren vor der Entwicklung des kombinierten SILAC-Verfahrens äußerst schwierig. Die neue Methode erlaubt nun einen Zugang zu diesen komplizierten experimentellen Forschungsansätzen.

Thomas E. Willnow

Structure of the Group

Group Leader
Prof. Dr. Dr. Thomas E. Willnow

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Dr. Tilman Breiderhoff
Dr. Anne-Sophie Carlo
Dr. Juliane Reiche
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Dr. Vanessa Schmidt

Graduate Students
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Molecular Cardiovascular Research

VPS10P domain receptors such as SORLA and sortilin comprise a recently identified class of intracellular sorting proteins that are predominantly expressed in neurons but also in non-neuronal cell types. VPS10P domain receptors were previously considered to be orphan receptors with activities in neuronal protein trafficking that were poorly understood. However, new findings revealed unexpected roles for these receptors as regulators of neuronal viability and function. Recent work from our laboratory has uncovered the molecular mechanisms of regulated protein transport and signaling through VPS10P domain receptors. Loss of this regulation may contribute to devastating disorders of the nervous system, including Alzheimer disease, affective disorders, and cell death following spinal cord injury. We also obtained first encouraging data concerning possible roles for these receptors in regulation of the cardiovascular system.

Introduction

The VPS10P domain is a protein module that was first recognized in the vacuolar protein sorting 10 protein (VPS10P) in *Saccharomyces cerevisiae*. VPS10P is a sorting receptor that directs the trafficking of lysosomal enzymes from the Golgi to the vacuole (the lysosome in *Yeast*). Subsequently, this protein domain was found to constitute the unifying structural feature of a new group of type 1-membrane receptors that are conserved throughout evolution from baker's yeast to man. The members of this gene family are now known as VPS10P domain receptors. Five receptors are found in vertebrates: sortilin, SORLA, SORCS1, SORCS2, and SORCS3 (Fig. 1A).

VSP10P domain receptors were initially considered a rather peculiar group of sorting proteins with unknown function. However, the mammalian receptors of the gene family surfaced as potential disease genes in a number of association studies in patients. These diseases encompass Alzheimer's disease (AD) and other types of age-related dementias (in which SORLA and SORCS1 have been implicated), bipolar disorders (in which SORCS2 has been implicated), as well as senescence of the nervous system (in which sortilin has been implicated). In addition, several common cardiovascular and metabolic disorders involving VPS10P domain receptors were identified including type 2 diabetes (which has been linked to SORCS1 and SORCS3) as well as dyslipidemia, and myocardial infarction (which have been linked to sortilin).

Subcellular trafficking of VPS10P domain receptors

The *trans*-Golgi network (TGN) is an organelle that is important for the distribution of proteins between various cellular compartments. It serves to direct newly synthesized proteins into constitutive or regulated secretion. It sorts proteins into endosomal or lysosomal compartments, and it participates in axonal transport and the action of signaling endosomes in neurons. Given its structural similarity to VPS10P, a sorting receptor in the TGN, a similar function for SORLA in Golgi trafficking had been anticipated. Our recent data on the

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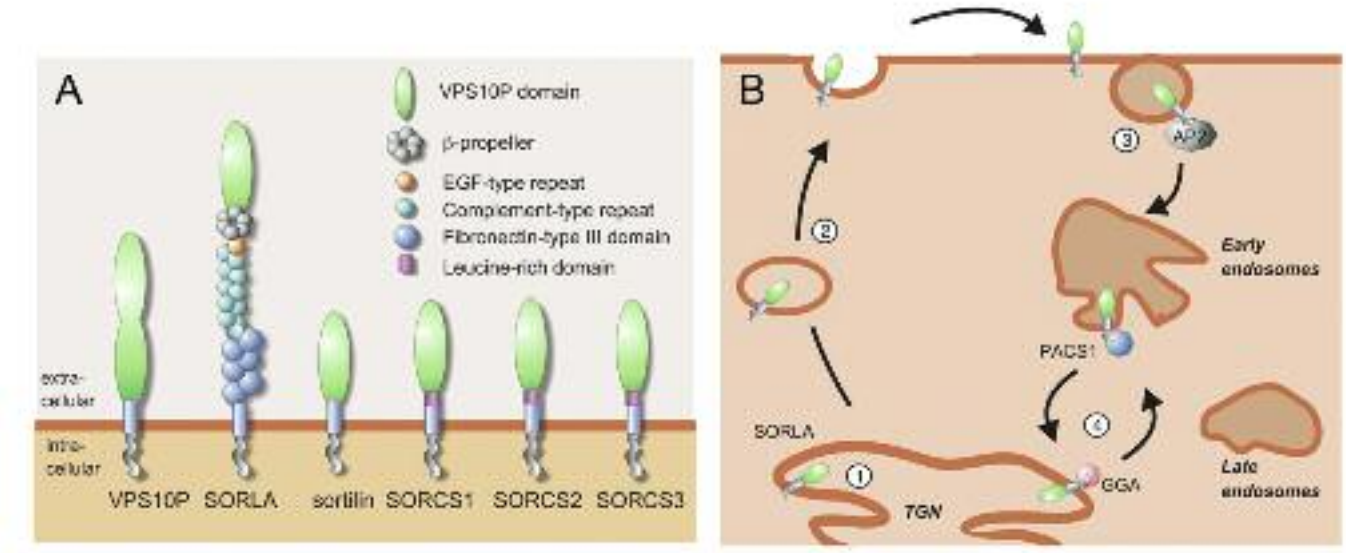

Figure 1: ***Structural and cellular biology of VPS10P domain receptors.*** *(**A**) Structural organization of VPS10P domain receptors from yeast (VPS10P) and humans (sortilin, SORLA, SORCS-1, -2, -3). (**B**) Cellular trafficking of SORLA. Newly synthesized receptors exit the trans-Golgi network (TGN) (1) and reach the cell surface via the constitutive secretory pathway (2). At the cell surface, they interact with the AP-2 complex to internalize via clathrin-coated pits (3). Internalized receptors are returned to the TGN via retrograde sorting pathways, and continue subsequent shuttling between endosomal and TGN compartments involving adaptor proteins GGA and PACS1 (4).*

cell biology of this receptor confirmed this notion (Fig. 1B). SORLA is predominantly found intracellularly in endosomal vesicles and the TGN. In neurons, the receptor is concentrated in the cell body with no apparent polarization. Receptor molecules at the plasma membrane exhibit rapid internalization that is mediated by an acidic cluster dileucine site in the cytoplasmic tail of SORLA that interacts with the ubiquitous adaptor complex AP-2. From early endosomes, internalized receptors are returned to the TGN (Fig. 1B). Using site-directed mutagenesis, we demonstrated that several cargo adaptors mediate the shuttling of SORLA between TGN and endosomes, including the clathrin adaptors GGA1, -2, -3 as well as PACS1 (Fig. 1B).

SORLA, a key gene in Alzheimer's disease

SORLA is a 250-kDa protein widely expressed in neurons of the cortex, hippocampus and cerebellum. Its involvement in Alzheimer's disease (AD) was initially suggested by the demonstration of low levels of *Sorla* gene expression in patients suffering from the sporadic form of the disease. The association of inherited *Sorla* gene variants with occurrence of AD in several populations further substantiated this notion. Now, our studies have uncovered the molecular mechanism whereby SORLA controls the intracellular transport and processing of the amyloid precursor protein (APP) and contributes to AD progression.

APP, a type-1 membrane protein is central to the pathology of AD. APP is converted to a 40 to 42 amino acid

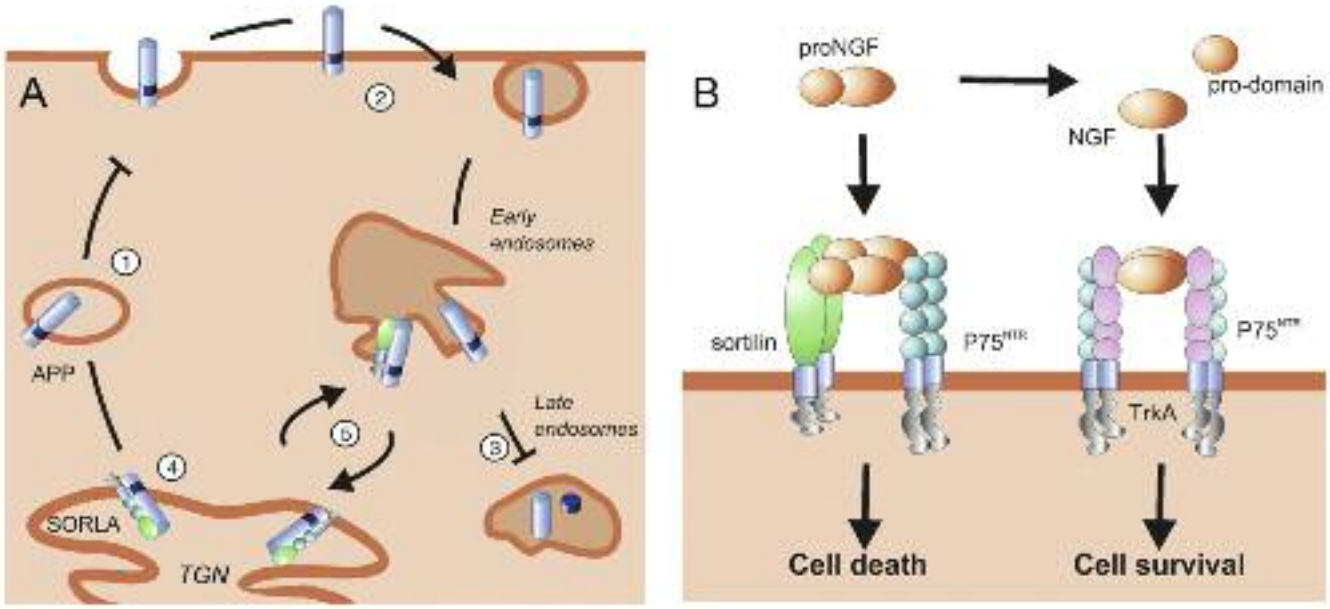

Figure 2: ***SORLA and sortilin in neuronal protein transport and signaling.*** *(**A**) Role of SORLA in trafficking and processing of APP. In neurons, newly synthesized APP molecules traverse the trans-Golgi network (TGN) en route to the plasma membrane (1). The precursor proteins internalize from the cell surface (2) and traffic from early to late endosomes for processing into Aβ (3). SORLA acts as sorting receptor that traps APP in the TGN, reducing the number of precursor molecules that can reach the cell surface and enter endocytic processing pathways (4). In addition, APP molecules that reach the cell surface and enter early endosomes are shuttled by SORLA back to the TGN, further reducing the extent of Aβ production in late endosomes (5). (**B**) Role of sortilin in (pro)neurotrophin action. Mature NGF binds to a heteromeric complex of TrkA and p75^{NTR} that stimulates signaling pathways promoting cell survival. In contrast, proNGF engages a dual receptor complex consting of sortilin and p75^{NTR} that elicits neuronal cell death.*

amyloid β peptide (Aβ) through sequential cleavage by β- and γ-secretases. In the brain, Aβ forms neurotoxic oligomers and amyloid plaques, the pathological hallmarks of AD. Using cell culture and transgenic mouse models we elucidated the mechanisms of SORLA action in neurodegenerative processes in molecular detail. According to our data, SORLA acts as intracellular sorting receptor for APP that controls trafficking of the precursor protein between TGN and early endosomes. In particular, SORLA blocks export of APP from the TGN into post-Golgi compartments that harbor β-secretase activity and thus impairs formation of Aβ (Fig. 2A). Consequently, genetic overexpression of SORLA in cultured neurons results in reduced processing of APP into Aβ. In contrast, disruption of the receptor gene in mouse models of AD significantly increased Aβ production and plaque formation, similar to the situation in patients with sporadic AD.

Sortilin controls neuronal cell death

As part of a collaborative project with the laboratory of Anders Nykjaer from Aarhus University, we uncovered the role of sortilin in the action of neurotrophins (NTs). NTs are growth factors that regulate neuronal survival, axon and dendrite specification, and target innervation. In mammals, the NT family includes nerve growth factor (NGF), brain-derived nerve growth factor (BDNF), as well as NT-3 and -4/5. Their trophic action is mediated by binding to receptor tyrosine kinases (called Trk receptors) and to the common p75 neurotrophin receptor (p75^{NTR}). All NTs are synthesized as proneurotrophins (proNTs) that are subsequently processed to their mature counterparts in the TGN. Paradoxically, while mature neurotrophins promote neuronal survival by binding to Trk receptors and p75^{NTR}, proneurotrophins induce apoptosis when released from cells.

Previously, we demonstrated that sortilin is an essential component for transmitting proNGF-induced death signals through p75 ^{NTR} (Fig. 2B). In this death receptor complex, sortilin specifically binds the pro-domain of the proneurotrophins with high affinity while p75^{NTR} simultaneously engages the mature NGF domain. The formation of this ternary receptor-ligand complex is crucial for the pro-apoptotic function. In this model, sortilin acts as the crucial molecular switch that enables neurons coexpressing p75^{NTR} and Trk receptors to selectively respond to proNGF by apoptosis rather than survival (Fig. 2B).

Now, we further substantiated the role of sortilin in proNT mediated cell death by generating a sortilin-deficient mouse model and by testing the contribution of the p75^{NTR}/sortilin receptor complex to neuronal viability *in vivo*. In the developing retina, *sortilin*$^{-/-}$ mice exhibit reduced neuronal apoptosis indistinguishable from that observed in *p75*$^{NTR-/-}$. Surprisingly, while sortilin deficiency does not impact on developmentally regulated apoptosis of sympathetic neurons, it prevents their age-dependent degeneration. Furthermore, in an injury paradigm lesioned corticospinal neurons in *sortilin*$^{-/-}$ mice are protected from death. Thus, the sortilin pathway plays distinct roles in proNT-induced apoptotic signaling in pathological conditions but also in specific stages of neuronal development and ageing.

Perspective

Dysregulation of vesicular protein transport is emerging as a molecular mechanism of major importance underlying many disease processes. Obviously, intracellular sorting receptors of the VPS10P domain receptor gene family play key roles in these processes. Our future work has yet to refine the molecular details how sortilin and SORLA affect neuronal viability and function through their interactions with neurotrophin receptors and APP. Furthermore, novel activities of the neuronal protein transport machinery may be uncovered as we learn more about the orphan receptors SORCS1, SORCS2, and SORCS3. As well as in the nervous system, SORLA and sortilin are also distinctly expressed in non-neuronal cell types such as in kidney, liver and adipose tissue. Our preliminary studies have uncovered important roles played by these receptors in renal ion transport and blood pressure regulation (SORLA) or in control of systemic cholesterol homeostasis (sortilin). In the future, we expect to gain novel insights into protein sorting pathways that may be central to the development of renal and metabolic disorders.

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Molecular Mechanisms in Embryonic Forebrain Development

Holoprosencephaly (HPE) is defined as a failure of the embryonic forebrain to separate into discrete hemispheres along the mid-sagittal axis. HPE is the most common forebrain anomaly in human embryos. Several pathways in neural tube patterning have been implicated in the etiology of HPE, including megalin (LRP2), a low-density lipoprotein receptor-related protein, expressed in the neuroepithelium during embryonic development. We have used megalin mutant mouse models to demonstrate that lack of megalin in the neuroepithelium causes HPE. Similarly, mutations in the human *Megalin* gene cause Donnai-Barrow syndrome in patients, characterized by facial dysmorphology, ocular anomalies, and agenesis of the corpus callosum – also features of holoprosencephaly. Our group is studying the mechanisms whereby megalin regulates signaling pathways crucial for early forebrain development. Our aim is to understand the pathophysiological pathways underlying common forebrain midline defects in patients.

Identification of defects in the molecular pathways underlying holoprosencephaly

(Annabel Christ, Oleg Lioubinski)

Megalin$^{-/-}$ mice suffer from forebrain defects with conditions associated with holoprosencephaly (HPE). Onset of the HPE phenotype becomes evident as early as mid-gestation. Megalin-deficient embryos at E 10.5 show significantly smaller telencephalic vesicles and an impaired subdivision of forebrain hemispheres.

To reveal the underlying molecular defects of the HPE phenotype in *megalin* mutant mice we analyzed the expression of marker genes involved in early forebrain development and found major changes in the expression and activity of key morphogens. We demonstrated that bone morphogenetic protein 4 (BMP4) signaling in the rostral and dorsal neuroepithelium is increased in *megalin*$^{-/-}$ embryos as early as E 9.5. Sonic hedgehog (SHH) expression is lost in the ventral telencephalon at E 10.5, while fibroblast growth factor 8 (FGF8) is aberrantly expressed in the rostral midline of the forebrain at E 9.5. Other pathways, e.g. the WNT signaling, do not show any obvious changes in *megalin* mutant mice.

Recent unpublished findings from our lab now shed light on the temporal onset of megalin function in forebrain patterning.

The mesodermal prechordal plate (PcP) anterior to the notochord underlying the rostral diencephalic ventral midline (RDVM) neuroepithelium in the forebrain is the essential organizing center for midline specification of brain, facial, and oral structures. SHH is secreted initially from the prechordal plate, subsequently the morphogen is found in the ventral midline of the forebrain neural plate where it induces its own expression and that of other factors involved in early forebrain patterning, e.g. the transcription factor *Six3*.

In our studies we detected SHH protein in the prechordal plate and the RDVM at E 8.25 (8 somites) in *wild type* mice whereas in *megalin* mutant embryos SHH was only found in the prechordal plate but not in the overlying neuroepithelium (Figure 1). It is only at the 10 somite stage (E 8.75) that the morphogen becomes detectable in the RDVM neuroepithelium of *megalin*$^{-/-}$ mice. This intriguing observation suggests that megalin deficiency delays secretion and proper distribution of

SHH. In line with these findings expression of Six3, a downstream target of SHH, is significantly down-regulated in the forebrain of *megalin*$^{-/-}$ embryos and *Shh* expression fails to be established properly in the ventral midline of the forebrain during later stages of development. It is likely that the delay and decrease in the signaling of SHH during the initial patterning of the ventral medial forebrain neural plate has detrimental consequences for CNS development in megalin mutants.

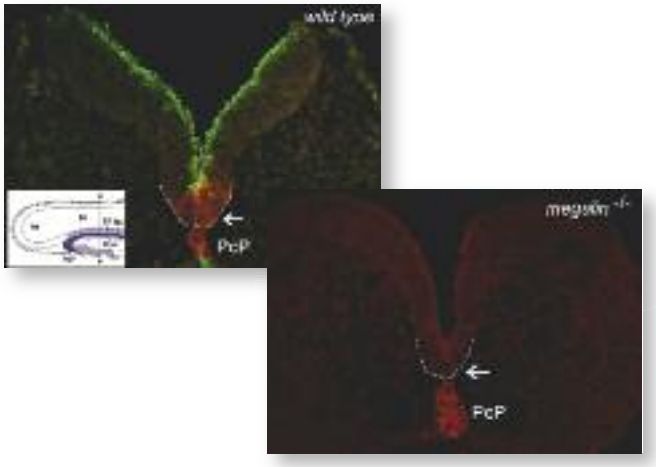
A role for megalin in adult neurogenesis

(Chandresh Gajera)

In the adult brain, megalin is expressed in ependymal cells, a specialized epithelial cell layer lining the brain ventricles. We found the strongest expression of the receptor in the lateral wall of the lateral ventricles compared to other ventricular regions. The lateral cell layer plays a crucial role in controlling the generation of adult neurons from neuronal precursor cells in the subventricular zone (SVZ) of the lateral ventricles. The SVZ is one of two regions of adult neurogenesis in the mammalian forebrain. Neuronal precursors generated in this region migrate to the olfactory bulb where they differentiate into mature neurons. Interestingly, SHH stimulates adult neurogenesis in the SVZ, and this effect requires repression of BMP activity via chordin and noggin, similar to the situation seen in the embryonic neural tube. Ependymal cells secrete noggin and thereby diminish the inhibitory effect of BMP4 on SVZ neurogenesis.

Recently, we obtained exciting new results from the analysis of adult megalin-deficient mice that provide direct evidence for a role of the receptor in adult neurogenesis. Megalin deficiency does not affect the cellular architecture of the SVZ in mice. Nevertheless, mutant animals show a decrease in cell proliferation in the lateral wall of the lateral ventricles (based on BrdU incorporation experiments) affecting the neuronal precursor population in the SVZ. The most prominent difference between megalin-deficient and control mice is found by staining for GFAP (glial fibrillary acidic protein), a marker for neuronal precursor cells. *Megalin*$^{-/-}$ mice show a weaker and altered staining pattern for GFAP in the SVZ. Furthermore, megalin mutant mice show a decrease in nestin, a marker of undifferentiated neuronal precursors, in Dlx2, a marker of rapidly dividing precursors, and in PSA-NCAM, a marker of migrating neuroblasts.

To address the mechanism whereby megalin influences the proliferation of neuronal precursors in the SVZ we tested the hypothesis that megalin may regulate the levels of neurogenesis by inhibiting BMP4 activity in the SVZ. We tested the expression of BMP2/4 and



Abnormal SHH protein expression in megalin mutant embryos
Immunostainings for Sonic hedgehog (SHH, red color) demonstrate expression of the morphogen on coronal sections of wild type mice at E 8.25 in the prechordal plate (PcP) and in the overlying rostral diencephalic ventral midline (RDVM) neuroepithelium (arrow, boundary indicated by the dotted line). Megalin protein is localized on the apical surface of the neuroepithelium (green color) in wild type embryos. In somite matched megalin deficient embryos SHH protein could be detected in the prechordal plate but SHH expression fails to be established in the overlying neuroepithelium during this stage of development. The inset shows a schematic illustration of the developing CNS at E 8.0 in a sagittal view with the mesoderm derived prechordal plate (PcP, purple) and the more caudal notocord (No) underlying the neuroepithelium of the diencephalon (Di) and the floorplate (FP), respectively. Te: telencephalon, End: endoderm

downstream mediators of this pathway. In these experiments, we discovered that there are increased levels of BMP2/4 protein specifically in SVZ in megalin mutants as compared to controls. We also detected a robust up-regulation of phospho-Smad 1/5/8, and ID3, downstream mediators and targets of BMP signaling, respectively. Taken together, these results provide conclusive evidence that megalin is a major regulator of adult neurogenesis in the SVZ, acting by suppressing the BMP4 signaling pathway. Remarkably, a similar function (to suppress BMP4 to activate SHH-dependent neurogenesis) is also performed by the receptor in the neural tube, supporting the concept that adult neurogenesis in the SVZ recapitulates features seen during formation of neurons in the embryonic brain.

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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. In striated muscle, Ca^{2+} bind to troponin C, which turn the thin filament "on", allowing myosin force-generating actin interactions. In smooth muscle cells, Ca^{2+} bind to calmodulin which then activate myosin light chain kinase. Phosphorylation of the 20kDa light chain of smooth muscle myosins then allows force-generating actin interaction. 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 striated and smooth muscle. Any change of these proteins by mutation, differential gene expression, alternative splicing of the transcripts, or post-translational modification modulate striated and smooth muscle function. Understanding muscle contraction regulation at the molecular and functional levels provide the opportunity to develop new therapeutic strategies for the treatment of cardiovascular and skeletal muscle dysfunction.

Essential myosin light chain (ELC) functions in the heart

Daria Petzhold, Janine Lossie, Maria Böhmer, Burcu Simsek; Ralf Meißner, Petra Sakel, Saskia Reichert

In the adult human heart two ELC isoforms are expressed, namely an atrial-specific (MYL4, ALC-1, accession NP_001002841) and a ventricular-specific (MYL3, VLC-1, accession NP_000249) isoform. ELCs bind with their N-terminus to actin and with their C-Terminus to IQ1 of the myosin lever arm (Figure 1). ALC-1 is preferentially targeted into sarcomeres of human and rodent cardiomyocytes. Most patients with hypertrophic cardiomyopathy and congenital heart diseases re-express hALC-1 in their ventricles, partially replacing the VLC-1 isoform. The VLC-1-to-ALC-1 isoform shift induced a pronounced positive inotropic effect.

The molecular basis for the isoform-specific sarcomeric sorting pattern and the molecular mechanisms of ALC-1 inotropy are not yet understood. In this project we test the hypothesis that different binding affinities of the C-terminus of essential myosin light chain (ELC) isoforms to the IQ1 motif of the myosin lever arm provide a molecular basis for distinct sarcomeric sorting and inotropic activity.

Hypertrophic Cardiomyopathy associates with five mutations in the essential ventricular myosin light chain gene (MYL3, AC_000135) (M149V, E143K, A57G, E56G, R154H). The pathomechanism of MYL3 mutations, however, is not yet understood. In this project, we will investigate the functional consequences of MYL3 mutations. We employ analytical ultracentrifugation, circular dichroism, and surface plasmon resonance spectroscopy to investigate structural properties, secondary structures, and protein-protein interactions of recombinant head-rod fragments of cardiac β-myosin heavy chain and ELC isoforms. Cellular functions of ELC isoforms will be investigated by monitoring shortening and intracellular free Ca^{2+} (Fura-2) of adult rat cardiomyocytes infected with adenoviral (Ad) vectors using ELC isoforms or β-galactosidase as expression cassettes. We will generate transgenic mouse lines overexpressing normal or mutated ELC isoforms. In addition, we elaborate a structural model which explains the cis-inhibitory action of the IQ2 domain on myosin function.

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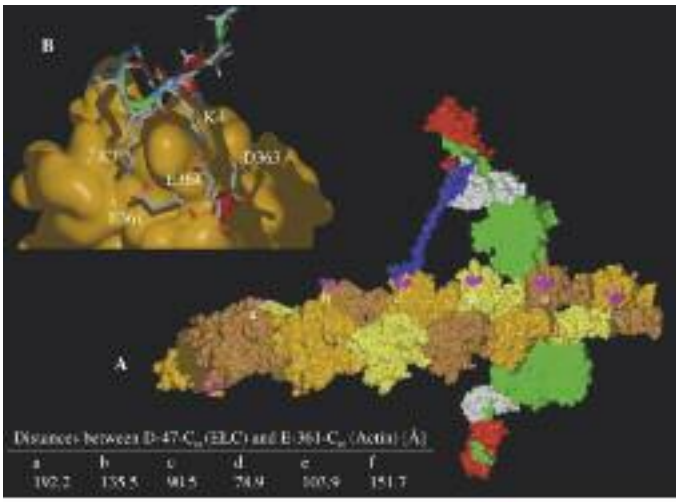

Figure 1. *3D-model of the actomyosin complex. (A) Gauss–Connolly surfaces are used to visualize the molecular complex. Actin units are coloured orange, brown, and yellow. The myosin S1 head (green), the regulatory light chain (red), the shortened essential light chain (white), the 46 N-terminal residues 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.*

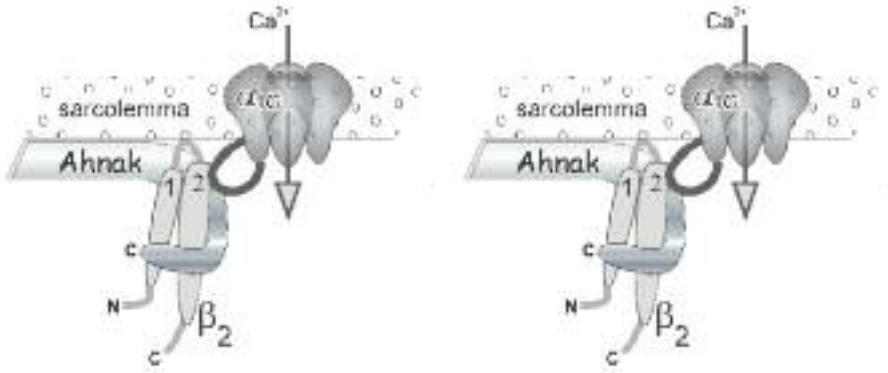

Figure 2. *Proposed model for sympathetic control of I_{CaL} by ahnak1. Under basal conditions, I_{CaL} carried by the α1C-subunit is repressed by strong ahnak1/β2-subunit binding (left panel). Upon sympathetic stimulation, PKA sites in ahnak1 and/or in β2 become phosphorylated. This releases the β2-subunit from ahnak1 inhibition resulting in increased I_{CaL}.*

The role of myomesin missense mutations on the genesis of hypertrophic cardiomyopathy

Romy Siegert (In collaboration with Cemil Öczelik, University Medicine Charité, Berlin and Irina Agarkova, Universitäts-Spital, Zürich,)

Hypertrophic cardiomyopathy (HCM) is a common cause of sudden cardiac death in young people. Three missense mutations in the myomesin gene have recently been detected in patients with HCM. These mutations are located close to the myosin and titin binding sites and the dimerization region. The aim of the project is to study the molecular pathomechanisms causing the development of HCM by myomesin mutations (e.g. V1490I). Therefore, functional properties of mutated recombinant fusion proteins will analyzed, i.e. force measurements of poly-myomesin by atomic force microscopy, structural properties by circular dichroism and melting curves, and cellular functions by transient expression of normal and mutated myomesin domains in cardiomyoblasts. Transgenetic rat lines which overexpress functionally relevant myomesin mutations will be generated in order to investigate their potential to cause HCM.

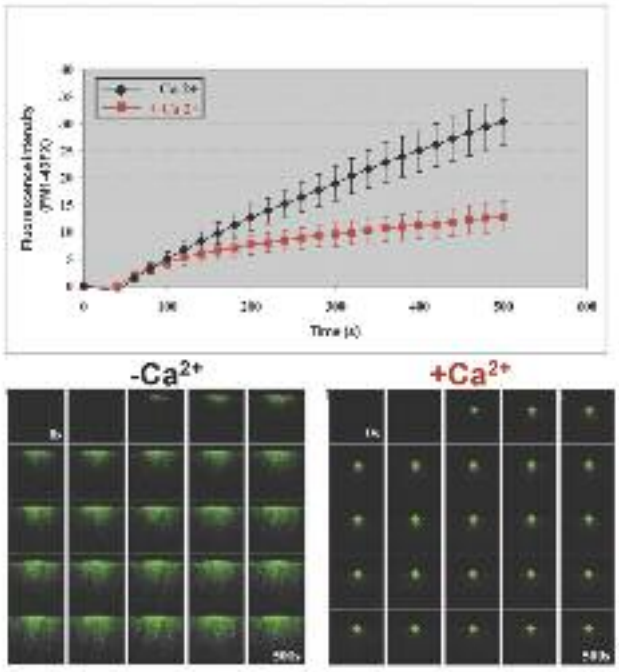

Figure 3. *Membrane resealing assay performed on wild-type mouse single skeletal muscle fibres. Membrane damage (5x5µm) was induced with a two-photon confocal laser-scanning microscope (LSM 510 META, Zeiss) coupled to a 488-nm Argon Ti:Sapphire Laser in the presence of FM 1-43FX fluorescence dye.* ***Top:*** *Plot of fluorescence intensity (n=10) against time in the presence (red line) and absence (black line) of Ca^{2+}. Data are means +-SEM.* ***Bottom:*** *fluorescence obtained in the presence (right) and absence (left) of Ca^{2+}.*

Ahnak1 – a novel, prominent modulator of cardiac L-type Ca^{2+} channels

Hannelore Haase, Ines Pankonien, Karin Karczewski, Steffen Lutter (In collaboration with Nathan Dascal, University Tel Aviv, Israel)

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. The goal of this study will be understanding the interactions of ahnak1 with L-VDCC, i.e. α1C and β2, and their modulation by β-adrenergic stimulation, mutations, and PKC activation (Figure 2). This will be achieved by heterologous expression of ahnak1 fragments in Xenopus oocytes and HEK cells and the characterization of their effects on cardiac and smooth muscle L-VDCC. Furthermore, reconstitution of β-adrenergic modulation of L-VDCC in heterologous expression systems, and elucidation of the role of ahnak1, Cavβ and other signaling components in PKA and PKC modulation will be studied.

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

Andreas Marg, Petra Domaing

The aim of the project is the elucidation of the role of ahnak protein family in skeletal muscle fibers. The transmembrane protein dysferlin seems to anchor ahnak1 and ahnak2 to the sarcolemma, thus providing a membrane-stabilizing dysferlin-ahnak-actin complex. We investigate whether the ahnak protein family is important for membrane stability and Ca^{2+} handling of skeletal muscle fibers. A laser-assisted membrane resealing (Figure 3) and a Fura2-based fluorescence assay of electrically stimulated enzymatically isolated single skeletal muscle fibers from mouse Flexor digitorum brevis will be applied.

Identification of new adipocyte-derived cardiodepressant factors

Christiane Look, Lena Martin (In collaboration with Valeria Lamounier-Zepter, University Dresden)

Recently, we showed that cultivated human adipocyte secrete factors which depress contraction of cardiomyocytes (Figure 4). The aim of this project is to identify adipocyte-derived cardiodepressant factors. We will assess the role of different adipose tissue depots in producing these cardiodepressant factors in our in vitro adipocyte/cardiomyocyte and adipocyte/isolated perfused heart models. Finally, the adipocyte-derived cardiodepressant factors will be analysed in vivo in microdialysis samples of subcutaneous adipose tissue of obese patients as well as in serum of our obese patient cohort. The resulting data will allow the development of risk profiling and novel therapeutic strategies for heart dysfunction in obese patients.

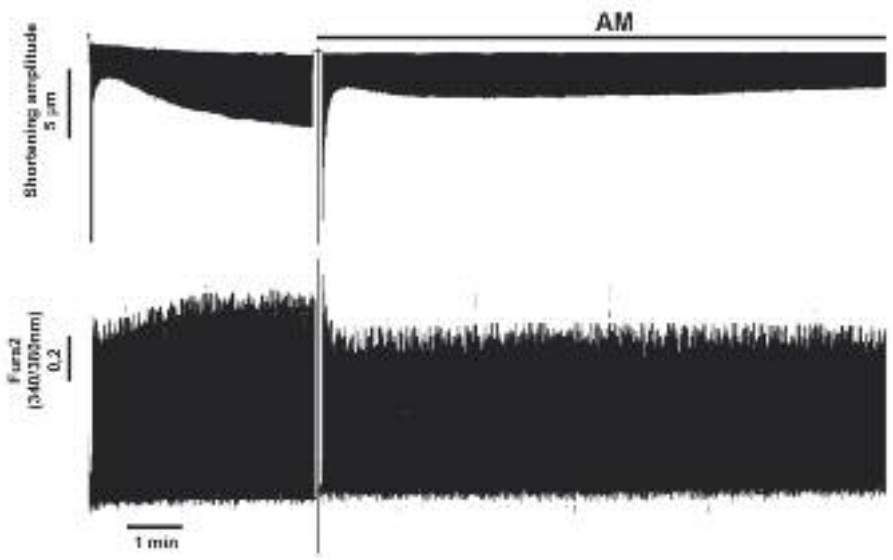

Figure 4. *Effect of adipocyte-conditioned medium (AM) on shortening amplitude (top trace) and Fura-2 signal (bottom trace) of an adult rat cardiomyocyte. A, B) Representative chart recordings of shortening amplitudes.*

Selected Publications

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Patent applications

„Behandlung der Dysfunktion der Herzkontraktilität oder Herzinsuffizienz, insbesondere bei übergewichtigen und adipösen Patienten mittels Hemmung von FABP4“. P1880EP

Michael Gotthardt

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Thirupugal Govindarajan
Katharina Rost*

Neuromuscular and Cardiovascular Cell Biology

Our long-term goal is to establish how mechanical input is translated into molecular signals. We focus on titin, the largest protein in the human body and the multi-functional 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. With these mice, we have demonstrated that CAR is crucial for embryonic development and determines the electrical properties of the heart.

Titin based mechanotransduction

Michael Radke, Thirupugal Govindarajan, Martin Liss, Padmanabhan Vakeel

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 and invertebrate giant titin-like molecules could act as a stretch sensor in muscle. More recently, this concept has been supported by studies on human dilative cardiomyopathies which suggest an impaired interaction of titin with its regulatory ligands such as Tcap. 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 protein expression and localization in our titin kinase and spring element deficient animals are used to map the mechanotransduction pathway.

Sarcomere assembly

Nora Bergmann, Katharina Rost, Thirupugal Govindarajan

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

Smooth muscle and non-muscle titins

Nora Bergmann, Katharina Rost

Recently titin has been proposed to perform non-muscle functions following its localization to various cell

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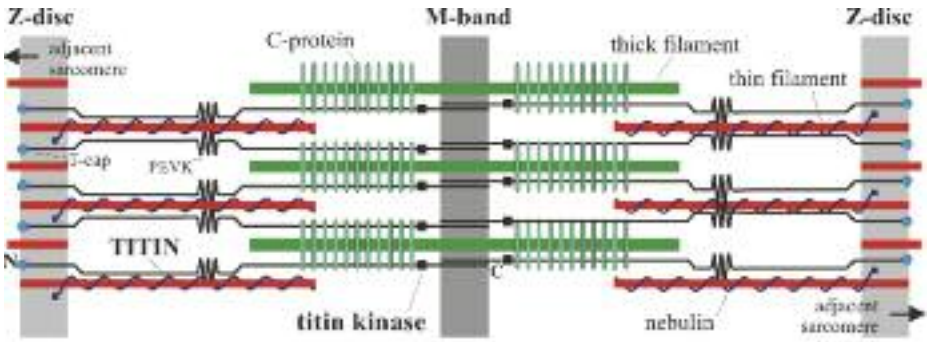
*part of the period reported
** guest, part of the period reported



Schematic diagram of the sarcomere. Titin forms a continuous filament system along the muscle fiber overlapping in the M-band (titin C-terminus) and in the Z-disc (N-terminus). The titin kinase is found near the edge of the M-band region, while the elastic PEVK resides in the I-band. Titin interacts with a plethora of sarcomeric proteins, such as T-cap and C-protein.

compartments such as the chromosomes of drosophila neuroblasts and the brush border of intestinal epithelial cells. Titin has been implicated in cytokinesis through localization to cleavage furrows and in chromosome condensation through localization to mitotic chromosomes. *Drosophila melanogaster* deficient in the titin homologue D-titin show chromosome abnormalities and aneuploidity.

Our preliminary data indicate that titin is present in virtually every cell-type tested. Nevertheless, our knockout of titin's M-band exon 1 and 2 does not show an obvious non-muscle phenotype, such as a defect in implantation or in cell-migration. Accordingly, we have extended the analysis of our titin knockout animals to actin-filament dependent functions (assembly of the brush border) and generated additional titin deficient animals to establish the role of titin in non-muscle cells.

Functional analysis of the Coxsackie-Adenovirus Receptor

Yu Shi, Chen Chen, Uta Wrackmeyer, Ulrike Lisewski

CAR was cloned as a receptor used by adeno- and coxsackievirus to enter cells but its physiological role has remained obscure. Detailed information on the expression pattern such as upregulation surrounding myocardial infarction and a critical role in embryonic development (lethality in mid-gestation of the CAR knockout) are well established, but no information on its role in the adult heart has been available.

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 preliminary 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.

Selected Publications

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Salim Seyfried

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Epithelial Morphogenesis and Zebrafish Genetics

Vertebrate organs are derived from epithelial sheets that undergo complex morphogenetic transformations. The molecular mechanisms that regulate the polarization of epithelial cells are crucial in this process. We have studied the early zebrafish heart, a relatively simple organ compared with its mammalian counterpart, to better understand the link between cell polarity, epithelial morphogenesis and signaling events that instruct the assembly of a heart tube. We would like to understand: How do the different protein complexes that establish cell polarity interact with each other? How is cell polarity regulated within epithelial sheets during morphogenesis of tissues and organs? What are the signals that regulate cell polarity and epithelial morphogenesis during cardiogenesis? Our long-term interest is to understand how the cellular mechanisms controlling cell polarity shape our own bodies. Insight from developmental genetics and cell biological approaches deployed within our research group are being used in collaboration with clinical research teams to identify genes responsible for human congenital heart disease.

Asymmetric behaviors of myocardial cells drive zebrafish heart tube formation

Stefan Rohr, Cécile Otten

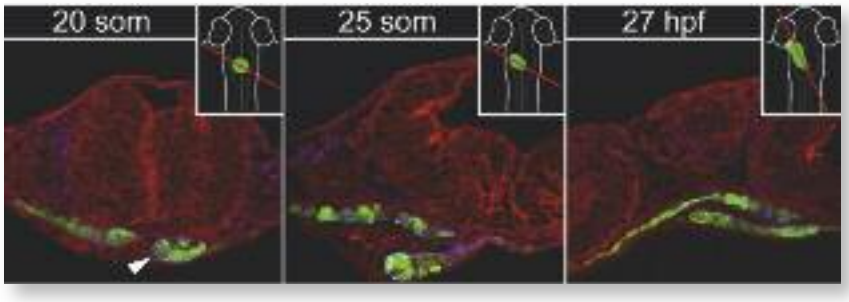
Many vertebrate organs are derived from monolayered epithelia that undergo morphogenetic changes to acquire their final shapes. Little is known about the tissue movements or cellular dynamics underlying early cardiac morphogenesis. In particular, the process by which the flat heart field is transformed into a linear tube was largely unexplored in vertebrates. In a recent study, we described a completely unexpected tissue morphogenetic process by which the nascent heart tube is generated in the zebrafish embryo. We discovered that asymmetric involution of the myocardial epithelium from the right side of the heart field initiates a complex tissue inversion which creates the ventral floor of the primary heart tube whereas myocardial cells derived from the left side of the heart field contribute exclusively to the future dorsal roof of this organ (Fig. 1). Intriguingly, asymmetric left-right gene expression within the myocardium correlates with asymmetric tissue morphogenesis and disruption of left-right gene expression causes randomized myocardial tissue involution. Our results demonstrated that asymmetric morphogenetic movements of the two bilateral myocardial cell populations generate different dorso-ventral regions of the zebrafish heart tube. Failure to generate a heart tube did not affect the acquisition of atrial versus ventricular cardiac cell shapes. Therefore, establishment of basic cardiac cell shapes precedes cardiac function. Together, these results provide a framework for the integration of single cell behaviors during the formation of the zebrafish primary heart tube.

Divergent polarization mechanisms during vertebrate epithelial development mediated by the Crumbs complex protein Nagie oko/Mpp5

Nana Bit-Avragim, Nicole Hellwig, Franziska Rudolph

(in collaboration with Chantilly Munson, Didier Y.S. Stainier, University of California, San Francisco)

Nagie oko (Nok)/Mpp5 is a member of the conserved Stardust/Pals1/Mpp5 family of MAGUK proteins and an integral component of the apical Crumbs-Patj-Par6-aPKC protein complex of cell polarity regulators. We performed a functional characterization of its different protein-pro-



Transformation of the flat heart field into an elongated heart tube. *Myocardial cells (green) of the right heart field initiate a tissue involution process which creates the ventral floor of the nascent heart (indicated by white arrow) whereas cells on the left side do not change their monolayered organization and form the dorsal roof. Various tissues are recognized by aPCK (red) and ALCAM (blue) staining. Inserts indicate the orientation of the section planes shown.*

tein interaction domains and obtained several surprising results that change the understanding of the extensive biochemical and *in vitro* protein-protein interaction data that have previously been described for this protein complex. Therefore, alternative scaffolding interactions must be in place, in addition to the *canonical* model of protein-protein interactions, to allow association of the larger Crumbs-Nagie oko-Patj-Lin-7-Par6-aPKC protein complex *in vivo*. Moreover, we provided evidence for different mechanisms involved in the polarization of the apical Crumbs complex within different tissues of the zebrafish embryo. These findings suggest that distinct epithelia employ divergently composed cell polarity complexes during tissue polarization.

Epithelial functions of aPKCi and Nagie oko/Mpp5 during cardiac morphogenesis

Stefan Rohr, Nana Bit-Avragim

Nok/Mpp5 is an essential scaffolding partner for both Crumbs and for the Par6-aPKC protein complex. In this study, we characterized early cardiogenesis in *heart and soul (has)/aPKCi* or *nok/mpp5* mutants. Loss of either of the two cell polarity regulators disrupted the epithelial organization of myocardial cells during heart tube formation and blocked the progression of cardiac morphogenesis beyond the heart cone stage. Has/aPKCi and Nok/Mpp5 were shown to function autonomously within the myocardial layer during morphogenesis and the catalytic kinase activity of Has/aPKCi was essential for this process. These findings demonstrated the importance of correct cell polarity and epithelial morphogenesis during heart formation.

Na^+,K^+-ATPase interacts with the apical Crumbs complex in maintaining myocardial polarity

Elena Cibrián-Uhalte
(in collaboration with Adam Langenbacher, Xiaodong Shu, Jau-Nian Chen, University of California Los Angeles)

In another study, we demonstrated the importance of correct ion balance for junctional maintenance and epithelial character of myocardial cells. The Na^+,K^+-ATPase, or Na-pump, is an enzyme primarily involved in the generation of Na^+ and K^+ gradients across membranes thereby regulating ionic concentrations within epithelial cells. The zebrafish 1B1 subunit of Na^+,K^+ ATPase is encoded by the *heart and mind* (*had*) locus and *had* mutants show a delayed heart tube elongation. This phenotype is reminiscent of the *has/aPKCi* or *nok/mpp5* mutant phenotypes which are characterized by a lack of epithelial cell polarity. In genetic interaction studies, Had/Na^+,K^+-ATPase and Nok/Mpp5, a component of the apical Crumbs protein complex, interacted in the maintenance of apical myocardial junctions raising the intriguing possibility that the ion balance produced by the Na-pump is critical for cell polarity. To functionally characterize the role of the ion pump function, we produced a mutant form of Had/Na^+,K^+ ATPase which specifically affects the ATPase activity that is essential for pumping sodium across the plasma membrane and found that it could neither rescue the heart tube elongation phenotype nor myocardial polarity. Our study suggests that the osmotic balance produced by the Na-pump contributes to the maintenance of apical junction belts, a function that is uncovered upon loss of Nok/Mpp5.

Selected Publications

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Ferdinand le Noble

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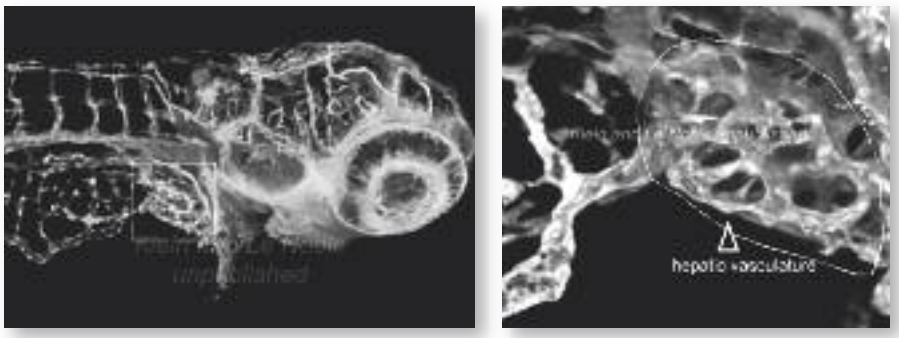
Angiogenesis and Cardiovascular Pathology

Vascular network remodeling and the formation of new blood vessels (angiogenesis and arteriogenesis) plays an important role in the pathophysiology of ischemic cardiovascular disease, hypertension, and cancer, which are the most common causes of mortality in western society. Our goal is to generate novel genetic insights in the regulation of vascular development that can translate into therapeutic strategies. Our research projects aim at understanding the molecular regulation of angiogenesis and arteriogenesis. We focus on three crucial aspects: differentiation and guidance of angiogenic vessel sprouts by endothelial tip cells, imprinting of arterial-venous identity in blood vessels by neural guidance genes and hemodynamic factors, and the formation and adaptation of native collaterals in the context of ischemic diseases. Using an integrative molecular and physiological approach in zebrafish, xenopus, and mouse, we want to investigate in which way manipulation of guidance molecules can be therapeutically relevant.

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: a) the specification of arterial and venous identity in vessels, b) the differentiation and guidance of endothelial tip cells in angiogenic vessels, c) the formation of branches and network patterning.

We discovered that neural guidance genes expressed in the vascular system control vessel branching morphogenesis by regulating the movement of endothelial tip cells at the leading edge of angiogenic vessel sprouts. We demonstrated that delta-like 4 Notch signaling controls the differentiation of endothelial cells into tip cells in response to VEGF gradients. The recognition that branching morphogenesis is controlled by a single cell type, the tip cell, that can sense attractant and repulsive signaling cues opens novel therapeutic avenues. Our current research endeavours therefore focus at elucidating the transcriptome and proteome of endothelial tip cell differentiation and function. We identified several novel candidate molecules involved in endothelial tip cell differentiation and vessel guidance events. We performed loss and gain of function experiments in zebrafish and mouse models to elucidate the mechanism of action of these molecules. Our candidates appear to directly affect tip cell differentiation from endothelial cells and coordination of their movement in the extracellular matrix. Interestingly, the guidance function of several of these navigation molecules also appears to play a pivotal function in organogenesis of heart, and liver. In this context we provided the proof of concept that in pathological conditions interference with guidance molecules maybe beneficial to prevent the development of dilated cardiomyopathy in hypoxic hearts.



Left panel: in vivo imaging of blood vessels and organ development in transgenic zebrafish expressing GFP in developing blood vessels. Right panel: detail of developing hepatic vasculature. (Klein&LeNoble, 2009, unpublished)

Mechanosensing, Arteriogenesis and Ischemic Diseases

Arteriogenesis, the outward remodeling of pre-existing small collateral arterial networks, occurs as a response to vascular occlusion or stenosis and importantly determines the clinical outcome of ischemic cardiovascular disease. Release of vasodilators and activation of inflammatory pathways allowing influx of monocytes may result in revascularization and restoration of blood flow into the hypoperfused ischemic area. Therapeutic arteriogenesis is considered of major clinical importance to treat the increasing population with complex occlusive artery diseases. Distinct differences exist between animal strains and patients with regard to collateral development and response to angiogenic growth factors. We aim at understanding the molecular mechanism accounting for such differences. In particular we focus on the formation of native collaterals and efficiency of collateral recruitment and maintenance. Hemodynamic forces exerted by flowing blood and neural guidance molecules play a critical role in initiation and maintenance of the arteriogenesis response. We are interested in how biophysical signals exerted by flowing blood activate specific genetic programs essential for arterial differentiation. The role of neural guidance molecules in arteriogenesis is studied in conditional mutant mice exposed to ischemic vascular stress (femoral artery occlusion, cardiac infarct, stroke models). We examined adaptive recovery from such pathological insults using laser-doppler flow, perfusion-microspheres, imaging including standard angiography, MRI and micro CT. In addition, we performed extensive molecular and histological analysis of target organs. Based on a comprehensive analysis of hemodynamic parameters in experimental models, and in vitro analysis we identified a specific component of the shear stress signal relevant for induction of arterial marker genes and arteriogenesis. At present we are characterizing the promoter elements triggered by shear stress relevant for arterial mechanosensing. Our data furthermore show that in mice native collaterals are already present at time of birth, but are progressively pruned during the course of postnatal life. Both the collateral numbers at birth, as well as their maintenance, are influenced by neural guidance gene function. The outstanding question is to what extent neural guidance gene function may account for the differences between "good" and "bad" collateralization responses as observed in clinical practice. In collaboration with clinical research partners we are evaluating novel strategies and compounds to stimulate arteriogenesis in patients suffering from vascular occlusive disease. In this context we now provided the proof of concept that, in humans suffering for ischemic heart disease, stimulation of shear stress using non-invasive external counter pulsation therapy, improves functional revascularization towards the ischemic regions.

Selected Publications

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Lu X*, Le Noble F*, Yuan L*, Jiang Q, De Lafarge B, Sugiyama D, Bréant C, Claes F, De Smet F, Thomas JL, Autiero M, Carmeliet P, Tessier-Lavigne M, Eichmann A (2004). The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. Nature 432(7014): 179-86. *equal contribution

Francesca M. Spagnoli

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** start of the group: July 2008

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Molecular and Cellular Basis of Embryonic Development

A fundamental question in developmental biology is how a specialized tissue originates from a pluripotent precursor cell in the embryo. The endoderm germ layer gives rise to a number of vital organs in our body, including the lungs, liver, pancreas and intestine. This remarkable diversity derives from a homogenous and multipotent precursor cell population. The central aim of our research is to understand the mechanisms that pattern and establish competence within anterior embryonic endoderm in order to progressively specify the pancreatic organ domain. In addition, we focus on spatio-temporal mechanisms that restrict specification of the pancreas versus neighboring tissues, such as the liver. A complete understanding of these early events will provide insights into the development of these organs. Finally, this information might be crucial for advances in regenerative medicine strategies for the treatment of incurable diseases, such as diabetes.

Projects

In vivo lineage analysis of pancreatic and hepatic precursor cells

Elisa Rodríguez Seguel, Igor Pongrac

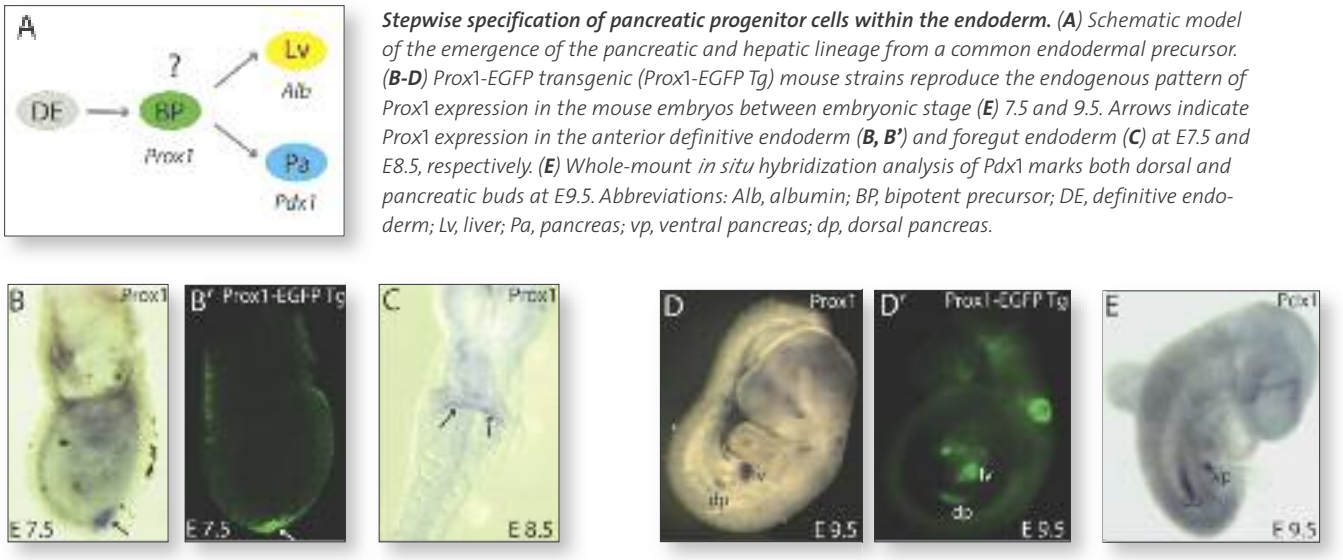
During embryogenesis, the pancreas originates from distinct outgrowths of the dorsal and ventral foregut endoderm. Both outgrowths give rise to endocrine and exocrine cells and, subsequently, fuse to form one single organ. The ventral pancreas arises next to the hepatic endoderm and they possibly originate from a common bipotent precursor. However, a single cell having the dual potential to differentiate along the hepatic and pancreatic lineages has never been isolated neither *in vivo* nor *in vitro*. One main focus of my laboratory is to investigate how pancreatic versus hepatic fate decision occurs in the endoderm at both the cellular and molecular level.

To conduct a comprehensive *in vivo* analysis of the hepatic-pancreatic lineage in the mouse embryo, we use transgenic reporter models that express EGFP or photoconvertible fluorescent proteins under the control of lineage-specific promoters. We are using these new genetic tools to: I. address *in vivo* and *in vitro* if the liver and pancreas arise from a common bipotent precursor; and II. to trace and molecularly profile the presumptive precursor cell and its descendants in the mouse embryo. All together, these experiments will determine how the hepatic-pancreatic lineage is established *in vivo*, whether a bipotent endodermal precursor exists, and provide us with its molecular signature.

Molecular mechanisms controlling pancreas versus liver fate decision

Nuria Cerdá-Esteban, Heike Naumann

Hepatic and pancreatic endoderm share a common set of intrinsic regulatory factors, such as the FoxA and GATA transcription factors, and are exposed to the same extrinsic signals, FGF and BMP. However, it remains unclear how the same factors can activate pancreatic genes, such as Pdx1 and Ptf1a, in the future pancreas, without inducing them in hepatic progenitors; and analogously, they enable liver development, but not pancreatic development in the hepatic endoderm. Both



Stepwise specification of pancreatic progenitor cells within the endoderm. *(**A**) Schematic model of the emergence of the pancreatic and hepatic lineage from a common endodermal precursor. (**B-D**) Prox1-EGFP transgenic (Prox1-EGFP Tg) mouse strains reproduce the endogenous pattern of Prox1 expression in the mouse embryos between embryonic stage (**E**) 7.5 and 9.5. Arrows indicate Prox1 expression in the anterior definitive endoderm (**B, B'**) and foregut endoderm (**C**) at E7.5 and E8.5, respectively. (**E**) Whole-mount in situ hybridization analysis of Pdx1 marks both dorsal and pancreatic buds at E9.5. Abbreviations: Alb, albumin; BP, bipotent precursor; DE, definitive endoderm; Lv, liver; Pa, pancreas; vp, ventral pancreas; dp, dorsal pancreas.*

intrinsic and extrinsic regulators of this cell fate decision are under study in my laboratory.

Our previous studies have elucidated the molecular events downstream of the GATA factors within the anterior endoderm in *Xenopus laevis*. We identified GATA downstream targets, such as TGIF2, acting as developmental regulators of the pancreatic versus hepatic fate decision. Interestingly, TGIF2 promotes pancreatic fate within the endoderm at the expense of hepatic markers. Conversely, in TGIF2-depleted embryos we observed expansion of the hepatic domain at the expense of pancreatic specification. We are currently investigating whether TGIF2 controls pancreatic versus hepatic fate decision and converts liver to pancreas upon over-expression in mammalian systems.

In parallel, we study how and when extrinsic factors, such as BMP, control the emergence of liver versus pancreas from the same embryonic region.

All together, these experiments will define developmental regulators that are able not only to specify one fate, but also antagonize the other (eg. pancreatic versus hepatic). This knowledge will be crucial for defining lineage reprogramming strategies of liver to pancreas toward a new cure for diabetes.

Novel signals guiding endodermal progenitors toward pancreatic fate

Kristin Petzold, Heike Naumann

In another line of research, we are investigating molecular players that act as instructive factors during early pancreatic development. In previous studies using the *Xenopus* system, we identified a novel pancreatic factor that we called Shirin. Very little is known about the biological function of this protein and, in particular, no embryological function has been assigned to it in mammalian species. We showed that Shirin is specifically expressed in the endoderm and pancreatic rudiments from gastrulation onwards, representing one the earliest marker of pancreatic endoderm. Gain-of-function experiments in *Xenopus* indicated that Shirin alone is sufficient to induce pancreatic identity, acting as a pancreatic instructive factor. Similarly, upon conditional ablation of Shirin gene expression in the mouse we observe defects in pancreas formation. Taken together, our observations suggest a conserved role for Shirin at very early stages of pancreatic specification.

At later developmental stages, Shirin is also expressed in neural crest territory and muscle progenitor cells. We are currently investigating a potential role of Shirin in these tissues in collaboration with the group of Prof. C. Birchmeier at the MDC.

Selected Publications

Spagnoli*, FM, Rosa*, A, Brivanlou, AH. (2009). Mesendodermal lineage specification in embryonic stem cells is under the control of a conserved microRNA family. Developmental Cell 16, 517-27. *co-first authors

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(Emmy Noether Research Group)

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Developmental Biology and Pathophysiology of the Kidney

The kidney is a central organ in cardiovascular diseases. It excretes toxins into the urine, regulates volume and solute homeostasis in the body, and produces hormones. The kidney is not only itself a target in cardiovascular disease and but also is centrally involved in cardiovascular homeostasis. Moreover, kidney failure constitutes one of the most important risk factors for other cardiovascular diseases. 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 nephron morphogenesis and the maintenance of epithelial integrity later in life. We focus on transcription factors and their regulation of aspects of epithelial differentiation. We use mouse models and epithelial cell culture systems and employ a wide spectrum of techniques, including genome-wide gene expression analysis, DNA-protein interaction analysis as well as organ culture techniques of the developing kidney.

Kidney Development

In the mammalian embryo, formation of the definitive kidney is initiated during mid-embryogenesis, when the ureteric bud, an epithelial tubule extending from the posterior Wolffian duct, interacts with an adjacent progenitor cell population, the metanephric mesenchyme. The ureteric bud 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. Our laboratory investigates the molecular mechanisms underlying these events, which are intriguing for several reasons.

First, 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 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.

Wnt signaling in Kidney Development and Disease

Wnt proteins are a family of secreted molecules that are important for various aspect of embryonic development. Several WNT molecules, including Wnt4, Wnt7b, and Wnt9b, are centrally involved in kidney development. We previously found that Wnt signaling via the β-catenin/TCF/Lef pathway mediates aspects of epithelial differentiation in metanephric mesenchymal progenitor cells. In addition, this pathway provides proliferative and antiapoptotic signals that regulate the size of the progenitor pool. As part of a DFG funded project (Schm 1730/2-1) we characterize the target gene program of

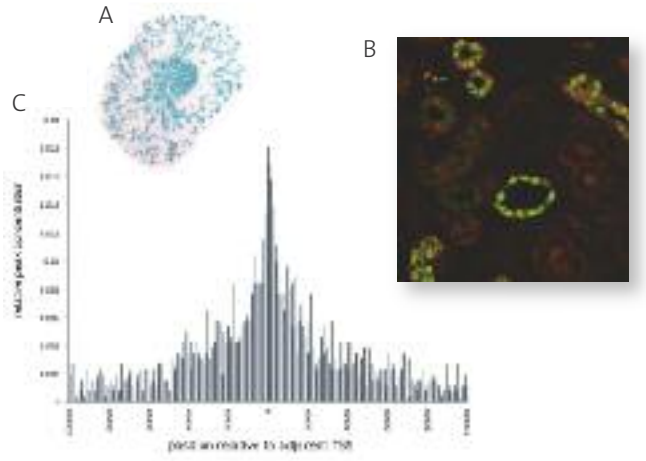
TCF/Lef signaling in the kidney. One crucial aspect of our future studies will be to elucidate the role of Wnt signaling in kidney disease, with an emphasis on the canonical TCF/Lef-dependent pathway. For this purpose we are using different genetic and experimental mouse models, which we analyze using genome-wide analysis of expression and transcription factor binding. We are working in close collaboration with Thomas Willnow (MDC), Friedrich C. Luft (ECRC), and Jonathan Barasch (Columbia University, New York). We believe that a detailed elucidation of the transcriptional network controlled by TCF/Lef will yield fundamental insights into growth, remodeling, and regeneration in the kidney.

Transcription factors involved in terminal renal epithelial differentiation

While TCF/Lef-dependent signaling accounts for early developmental programs in renal epithelial progenitors, it may in fact be inhibitory to terminal differentiation of tubular epithelia. Therefore, we are seeking complementary transcriptional regulators that induce the establishment of epithelial polarity and the expression of segment-specific markers. Using expression profiling, we identified two candidate transcriptional regulators of terminal differentiation, which belong to the CP2 group of transcription factors (Tcfcpl1 and Grhl2), which are highly and specifically expressed in the distal nephron. Characterization of Grhl2 is part of a DFG-funded project (Schm 1730/3-1) within FG "Epithelial mechanisms of renal volume regulation" (Speaker: Sebastian Bachmann, Charité Berlin). The projects benefit from our close collaborations with Jonathan Barasch (Columbia University, New York), Thomas Willnow (MDC), and Michael Bader (MDC).

Biomarkers 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), Friedrich C. Luft (ECRC) and Ralph Kettritz (ECRC), we are conducting clinical studies to test the performance of NGAL as a urinary and plasma biomarker of acute kidney injury. We are aiming to optimize diagnostic algorithms that utilize NGAL measurements to predict renal injury or to differentiate renal injury from related clinical entities. This project serves as a prime example to illustrate the importance of an understanding of basic molecular mechanisms in the embryo to address the diagnosis of renal disease in the adult. Translational aspects of our research are markedly enhanced by our close ties to the 'Experimental and Clinical Research Center' (see ECRC section).



Characterization of transcription factors in nephron epithelia. ***A.*** *β-Galactosidase expressed from the Grhl2 locus displays an epithelial-specific pattern in the early postnatal kidney.* ***B.*** *Nuclear localization of the transcription factor Tcfcp2l1 (green) in a subset of nephron epithelia (red) as detected by confocal microscopy.* ***C.*** *Transcription factor binding to genomic DNA in a renal epithelial cell line as determined by chromatin immunoprecipitation followed by massively parallel sequencing. The frequency plot reveals strongly enriched transcription factor binding to genomic DNA around transcriptional start sites (TSS).*

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Nikolaus Rajewsky

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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 the function of small RNAs during early development in *C. elegans* (Fig. 1). Furthermore, we have established planaria (Fig. 2) 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 the role of small RNAs 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 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, long-term memory, signaling, homeostasis of key metabolic gene products such as cholesterol, apoptosis, onset of cancer, Tourette's syndrome, and others. 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 also explore the role of RNA binding proteins.

Systems Biology of Gene Regulation

Catherine Adamidi, Kevin Chen, Minnie Fang, Marc Friedlaender, Signe Knespel, Azra Krek, Andreas Kuntzagk, Svetlana Lebedeva, Jonas Maaskola, Marlon Stoeckius, Nadine Thierfelder

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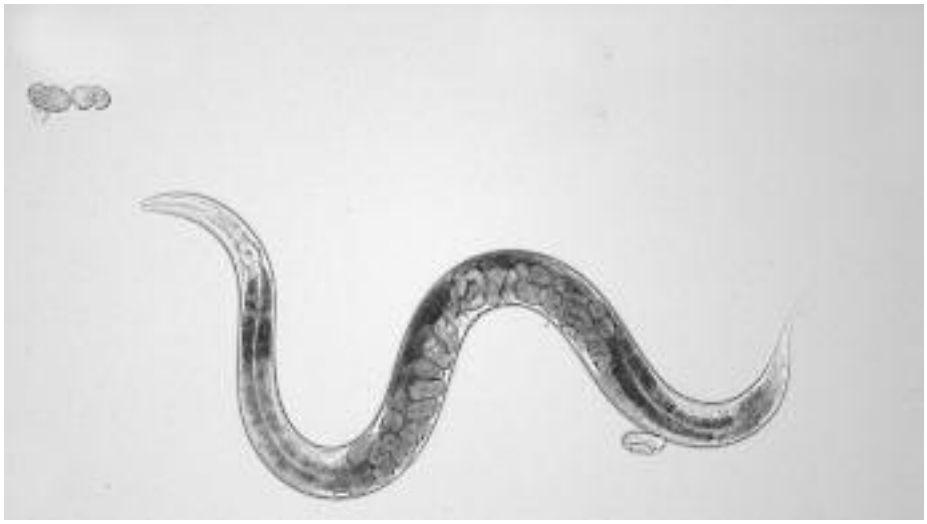

Figure 1. *C. elegans*

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 function, 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 gene 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*, and within collaborations with other experimental groups.

Function of microRNAs

(Azra Krek, Marc Friedlaender, Minnie Fang, Marlon Stoeckius, Jonas Maaskola, Nadine Thierfelder, Svetlana Lebedeva, Sebastian Mackowiak, Marvin Jens).

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. 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, and that human genotyped SNP data can be used to explicitly demonstrate and quantify the contribution of microRNA targets to human fitness. All microRNA target predictions of our algorithm PicTar can be accessed at our searchable PicTar website. We have further developed computational methods to predict miRNAs from high throughput sequencing data (see miRDeep webpage). We have also pioneered approaches that allowed

Figure 2. *Planaria*

to experimentally assay, genome-wide, the impact of miRNAs on protein synthesis (see pSILAC website). 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 if RNA binding proteins.

These projects involved or involve, in part, collaborations with the following labs: Markus Stoffel lab (ETH Zurich), Fabio Piano and Kris Gunsalus (NYU), Matthias Selbach (MDC), Markus Landthaler (MDC).

Early embryogenesis in *C. elegans*

Marlon Stoeckius, Jonas Maaskola, Nadine Thierfelder

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). However, high-throughput technologies are needed to solve several fundamental problems in embryogenesis, for example how post-transcriptional and later transcriptional regulatory networks drive development. One key problem is that the state of the art method to obtain precisely staged early embryos consists of sorting embryos via mouth pipetting, thus making it impractical to obtain large samples. 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 (Figure 3). 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 are proceeding to develop and use in vivo SILAC to study the functional impact of certain small RNAs on the post-transcriptional level. These projects involve collaborations with the F. Piano lab (NYU), M. Selbach (MDC), W. Chen (MDC) and others.

Small RNAs in planarian stem cell biology

Catherine Adamidi, Marc Friedlaender, Pinar Oenal, Sebastian Mackowiak

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 are currently starting to functionally follow up some of these results. We are also starting to systematically identify the proteome of planarian stem cells and are interested to use these data to identify and characterize key genes involved in regeneration mediated by

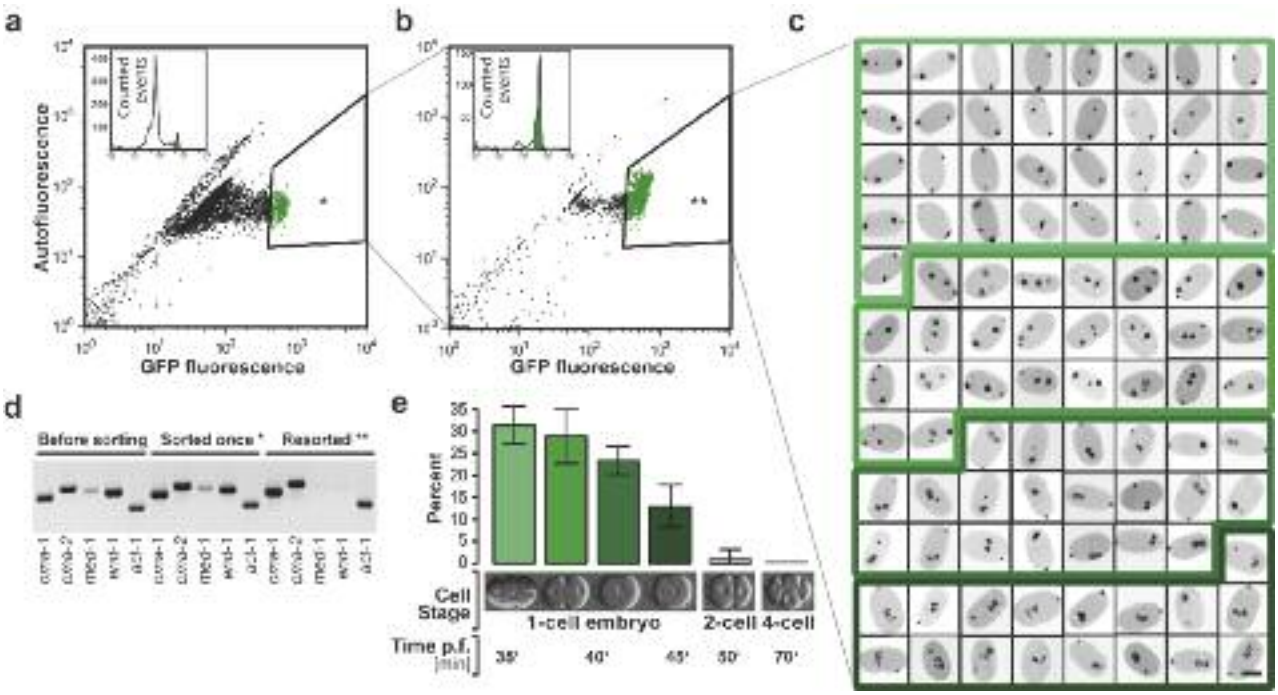

Figure 3. *Proof of principle: FACS sorting of a mixed C. elegans embryo population (eFACS) routinely yields tens of thousands of almost perfectly clean one-cell embryo population (Stoeckius & Maaskola et al, Nature Methods 2009). A transgenic strain that expresses GFP most highly in the one cell embryo is used. Embryos are fixed with Methanol, and the GFP positive population marked in green (a) is resorted (b). A random sample of 96 embryos from the resorted population consists to at least 98% of one cell embryos, as shown by microscopic analysis (c) and also confirmed by PCR markers (d).*

stem cells. These projects involve, in part, collaborations with the Sanchez lab (U. of Utah), S. Kempa (MDC), W. Chen (MDC) and others.

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Krek, A, Gruen, D, Poy, MN, Wolf R, Rosenberg L, Epstein EJ, MacMenamin P, da Piedade I, Gunsalus KC, Stoffel M, and Rajewsky N (2005). Combinatorial microRNA target predictions. Nature Genetics 37, 495-500

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Norbert Hübner

Structure of the Group

Group Leader
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Dr. Claudia Gösele
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Dr. Carola Rintisch
Dr. Franz Rüschendorf
Dr. Klaus Rohde
Dr. Kathrin Saar
Dr. Herbert Schulz

Medical Genomics and Genetics of Complex Cardiovascular Diseases

My group is investigating the molecular genetic basis of common cardiovascular risk factors and disorders in experimental rodent and human populations. We are working with inbred rat strains since they provide one of the most relevant models of common multifactorial cardiovascular human disease. It has been the major model for physiological investigation, providing a body of data on patho-physiology, including detailed mechanistic, biochemical and metabolic characterisation that cannot easily be replaced by other models. The work of our group focuses on the development of genomic tools and to utilize these for functional genetic and genomic approaches for complex disease gene identification. Additionally my group has an increasing interest in translating findings from model organisms to humans by comparative genome analysis.

Establishment of functional genetic and genomic resources for the rat

We have developed a large number of genomic resources to facilitate functional genomic studies in the rat. These efforts included long-range physical maps and characterization of single nucleotide variation within the rat genome. We contributed to the annotation and assembly of the rat genome sequence. The recent availability of the rat genome sequence and associated genomic tools has raised the profile and pace of research into genetic analysis of rat traits and dramatically accelerated prospects for gene identification. Decades of exquisite phenotyping and detailed analysis of crosses of inbred rats have resulted in initial localization of hundreds of loci involved in complex disease and quantitative phenotypes, but with very few eventual gene identifications to date. A clear understanding of the origin and structure of genetic variation in the rat will provide a key-missing piece of this puzzle. To fully realize the power of the recent rat genome sequence, we are currently initiating the complete genetic dissection of the ancestral segments making up the most commonly used inbred lines.

Haplotype mapping for genetic analysis in the rat

Genetic variation in genomes is organized in haplotype blocks and species-specific block structure is defined by differential contribution of population history effects in combination with mutation and recombination events. Haplotype maps characterize the common patterns of linkage disequilibrium in populations and have important applications in the design and interpretation of genetic experiments. Although evolutionary processes are known to drive the selection of individual polymorphisms, their effect on haplotype block structure dynamics has not been shown.

We have led an international consortium (STAR) and reported a survey of genetic variation based on almost 3 million newly identified SNPs. We constructed high-density genetic maps, creating a large dataset of fully characterized SNPs for disease gene mapping. Our data characterize the population structure and illustrate the degree of linkage disequilibrium. We provide a detailed SNP map and demonstrate its utility for mapping quantitative trait loci. This community resource is openly available and augments the genetic tools for studying this model of human disease.

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Genome approaches to dissecting cardiovascular and metabolic disease

The rat genome sequence together with other genomic resources, including genetic and structural variation maps, provide exceptional opportunities for identifying genes and pathways underlying disease phenotypes. Our collaborators and we have devised a systems approach for dissecting genetic networks systematically across the biological scale, from molecular to physiological, using segregating rat populations We combined linkage analyses with genome-wide expression profiling and identified independent genes contributing to heart failure susceptibility, increased left ventricular mass, and hypertension in spontaneously hypertensive rats (SHR) and SHR derived substrains. Moreover, we are interested in the genetic regulation of gene expression in a range of insulin sensitive tissues including fat, kidney, adrenal, heart, skeletal muscle, the vasculature, and liver in rat RI strains. Using the data collected across multiple tissues we can now detect the genotype dependent co-expression of gene networks and suggest their possible biological implications for common cardiometabolic disorders. 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.

Next generation sequencing of the spontaneously hypertensive rat genome

The genome sequence of the normotensive BN strain is available but only limited knowledge exists of genomic sequence variability between SHR and BN. Within an international collaboration we started to sequence the SHR genome using paired-end sequencing technique on the Solexa platform. Nearly 816 million reads (33.8 GB) were sequenced of which 87% of reads were mapped to the reference BN genome giving 10.7x coverage of the SHR genome. We identified 3.6 million high quality SNPs and 343,243 short indels (1 to 15 bp) between SHR and BN with a very low false positive rate (<1%). More than 80% of the identified SNPs were novel. Structural variations (SVs) between SHR and BN genomes were identified using read pairs that mapped incorrectly to the reference genome. We identified 20,298 SVs including 15,037 deletions, 4,861 insertions and 400 inversions. SVs ranged from 50 bp to 1 Mbp in length. Using read depth data of correctly mapped read pairs we identified 136 copy number variations (CNVs). 88 of the copy number variable regions cover 260 genes including the previously identified CNVs at Cd36 and Fcgr3. These data show that large number of genomic sequence variations including SNPs, SVs and CNVs can be identified with high resolution using paired end sequence data. These findings will greatly accelerate identification of genomic changes underlying hypertension and other complex phenotypes in the SHR strain.

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Friedrich C. Luft

Structure of the Group

Group Leader
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Mouse physiology
Volkmar Gross

Gene lab
Sylvia Bähring

Eicosanoid metabolism
Wolf-Hagen Schunck

Hypertension, Vascular Disease, Genetics, and Nephrology

The Department of Nephrology/Hypertension/Clinical Pharmacology, headed by Friedrich C. Luft (since 1992) encompassed numerous groups that are now represented independently elsewhere (Clinical Research Center, Ralph Kettritz, Dominik N. Müller, Maik Gollasch, Kai Schmidt-Ott). The core group has focused on genetic rearrangements as a cause of somatic alterations and cardiovascular disease. The group has also directed its attention to elucidating mechanisms of hypertension by means of novel mouse models. In addition, the group has also focused on alternative mechanisms of mineralocorticoid-induced vasculopathy. The group has elucidated the role of eicosanoids in arrhythmia generation and gap junction protein function. Finally, the group has collaborated in efforts directed at salt-sensitive hypertension and novel driving forces.

Molecular genetics

Sylvia Bähring leads "gene lab" a laboratory dedicated to clarifying complex genetic issues. She is not focusing on straight-forward mutations. The primary project involves autosomal-dominant hypertension with brachydactyly type E (BDE). We now know that these (we have 5 families) are complex rearrangement syndromes on Chromosome 12p. The inversion region for hypertension and brachydactyly on chromosome 12p features multiple splicing and noncoding RNA. We have successfully dissected the disease-carrying chromosomal bands on the short arm of 12p with help from collaborators in Jena, and will now subject this material to "next-generation" sequencing. Atakan Aydin is focusing on this important issue supported by the groups of Wei Chen and Norbert Hübner at the Genome Center. We showed earlier that our primary suspect (a gene with no open reading frames or Kozac sequences) codes for a microRNA that is responsible for the syndrome. We are expending most of our energies to elucidate this important project. Our efforts include a collaboration with Matthias Selbach and Nikolaus Rajewsky to express our putative microRNA in HELA cells in order to find out the target genes on RNA and protein level and to elucidate the functional pathways leading to the phenotypes.

Philipp Maass pursues another important project of our group. We studied a family BDE, albeit without hypertension. We found a t(8;12)(q13;p11.2) translocation with breakpoints upstream of *PTHLH* on chromosome 12p11.2 and disrupted *KCNB2* on 8q13. The *PTHLH* translated PTHrP protein regulates chondrocyte differentiation via a negative-feedback loop implicating the signaling factor IHH. We determined a *PTHLH* down- and *IHH* upregulation in chondrogenic induced fibroblasts from affected BDE patients. We sequenced the translocation breakpoints and found a conserved AP1 transcription factor binding site on chromosome 12p11.2 and an ETS1 binding site from 8q13, which resides near the AP1 site due to the translocation. We observed binding of both transcription factors at the breakpoint sequence. Since AP1 and ETS1 interact, we tested if these factors regulate *PTHLH* in murine and human chondrocyte cell lines. Furthermore chondrocytes and BDE fibroblasts showed different epigenetic modifications between the wild-type and breakpoint allele with an enrichment of histone modifications H3K4me1 and H3K4m3, which are

Mineralocorticoid receptor
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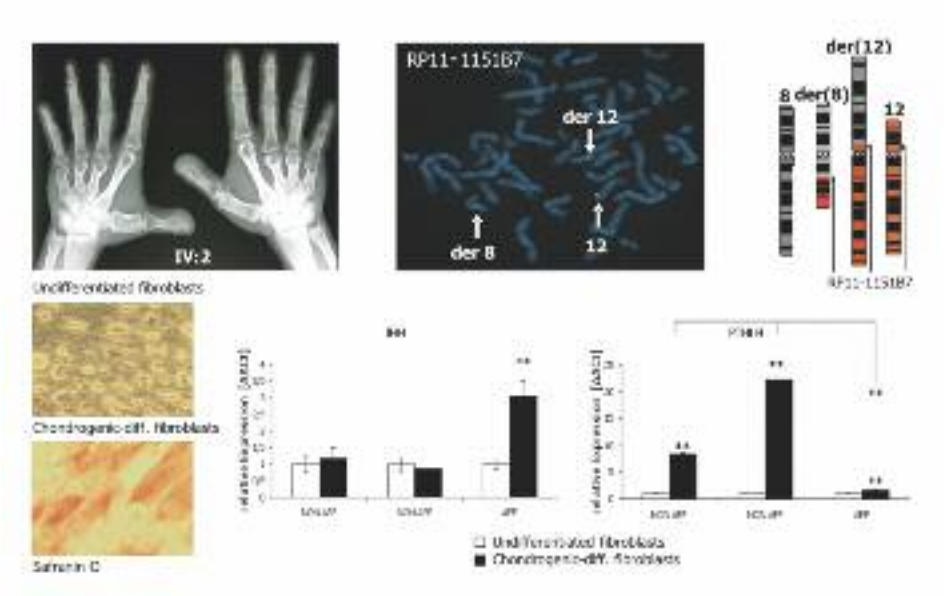

Figure 1. *Type E brachydactyly, balanced translocation t(8;12)(q13;p11.2) in Metaphase-FISH, fibroblasts transformed to chondrogenic cells, upregulation of Indian hedgehog (IHH) and downregulation of parathyroid-like hormone (PTHLH).*

generally associated with regulatory activity. Our findings have broader implications for understanding how chromosomal aberrations affect epigenetic modifications and long-range gene regulation. The results indicate that dysregulation of chondrogenesis due to a novel site for interaction of AP1 and ETS1 upstream of PTHLH, is the underlying cause for the BDE.

That is all very nice but it's mice

Volkmar Gross and Friedrich C. Luft have been collaborators for almost 20 years. They have maintained the standard of conservative cardiovascular physiology as opposed to descriptive RT-PCR, RNase protection assays, and Western blotting (where physiology ends by the others), although we have incorporated all these techniques to test our hypotheses. Our goal is to show it at the experimental level, gene expression can be no substitute. Gross and Luft have attempted to bring all of cardiovascular physiology to mice and particularly to unique mice. Recently, we investigated synophilin (a collaboration with Paul Greengard, Nobel Prize 2000). The spinophilin protein controls intensity/duration of G protein-coupled receptor signaling and thereby influences synaptic activity. We hypothesized that spinophilin affects blood pressure through central mechanisms. We measured blood pressure and heart rate in SPL-deficient -/-, SPL+/-, and SPL +/+ mice by telemetry combined with fast Fourier transformation. We found that an increase in central sympathetic outflow participates in blood pressure and heart rate increases in SPL -/- mice. The elevated blood pressure in SPL(-/-) mice was associated with attenuated baroreflex sensitivity and decreased parasympathetic activity. Our study is the first to show a role for the spinophilin gene in blood pressure regulation. These novel results have a bearing on centrally (brain) mediated control of blood pressure. The group has pursued additional mouse-related projects. These include work on db/db leptin receptor deficient mice and mice deficient in the solulble epoxide hydrolase.

Mineralocortcoid receptor signaling

Annete Fiebeler has led a group of investigators specifically interested in mineralocorticodi-reseceptor signaling. In a recent study, she and friends showed that corticosterone induces rapid mineralocorticoid receptor

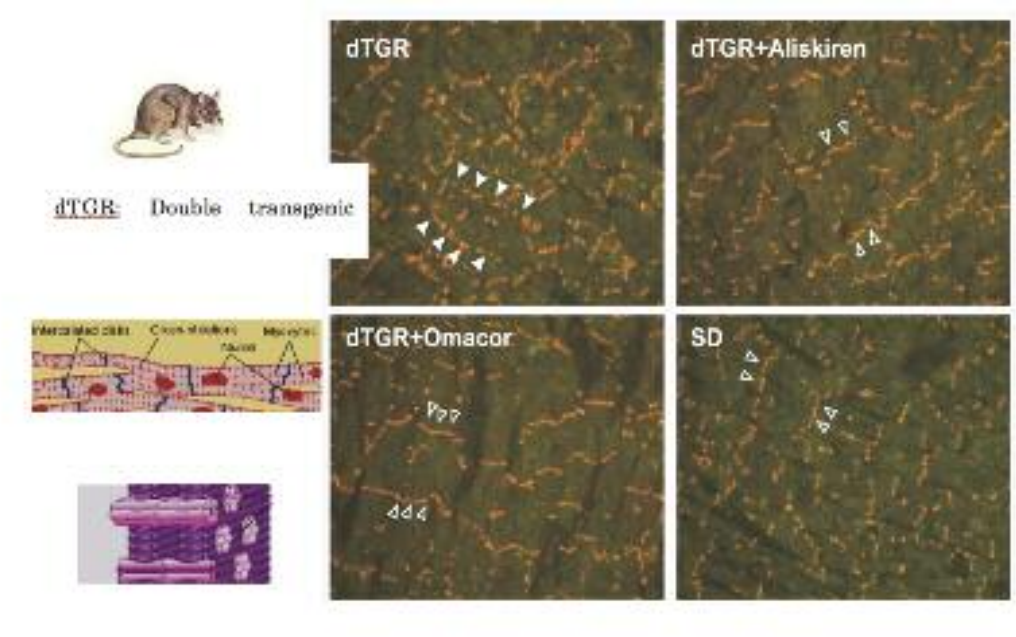

Figure 2. *Omega-3 fatty acids protect against connexin 43 dyslocalization and sudden cardiac death. Connexin 43 (Cx43), a mediator of intermyocyte conduction, is redistributed to the lateral cell membranes in the hearts of dTGR making them highly susceptible to fatal arrhythmias. Dietary omega-3 fatty acids (Omacor) and aliskiren normalized Cx43 localization to the gap junctions within the intercalated disks and protected the animals against sudden cardiac death.*

signaling in vascular smooth muscle cells that involves mitogen-activated protein kinase kinase/extracellular signal-regulated kinase-dependent pathways. These new mineralocorticoid receptor-dependent signaling pathways suggest that glucocorticoids may contribute to vascular disease via mineralocorticoid receptor signaling, independent of circulating aldosterone. More recently, the group showed that the growth arrest-specific protein 6 (GAS6) participates in desoxycorticosterone-induced (DOCA) hypertension and target organ damage. They found that Gas 6 was upregulated in rats with high aldosterone levels. Mineralocorticoid receptor blockade prevented target organ damage and decreased the elevated Gas 6 expression. Vascular smooth muscle cells given aldosterone increased their Gas 6 expression in vitro. Cardiac expression of interleukin 6 and collagen IV was blunted in Gas 6(-/-) mice, indicating reduced inflammation and fibrosis. Gas 6(-/-) mice also had an improved renal function with reduced albuminuria, compared to wild-type mice. Gas 6 appears to play a role in mineralocorticoid receptor-mediated target organ damage. Furthermore, because warfarin interferes with Gas 6 protein expression, the findings could be of clinical relevance for anticoagulant choices.

EETs, HETEs and alternatives
Role of enhanced EET-degradation in heart failure

Wolf-Hagen Schunck

A major route of EET-degradation is catalyzed by the soluble epoxide hydrolase (sEH), an enzyme that is upregulated by angiotensin II (Ang II) in the heart and vasculature. We collaborated with Jan Monti (ECRC), Norbert Hübner (MDC), and numerous others and showed that spontaneously hypertensive rats developing heart failure (SHR-HF) map to the gene locus for sEH (EPHX2). Monti and Hübner found a mutation in the EPHX2-promoter. We used sEH -/- mice and material from the rats and showed that this mutation directly affects sEH-expression and cardiac EET-levels. We further showed that sEH -/- mice were relatively resistant to Ang II- and pressure overload-induced heart failure and cardiac arrhythmia. This project involving collaboration between MDC basic science and ECRC clinicians is an excellent example of translational medicine.

Role of omega-3 fatty acid derived CYP-eicosanoids in cardiac arrhythmia

Our team collaborated with Robert Fischer and Dominik Müller and demonstrated that eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) protect against

arrhythmia and sudden cardiac death. Dietary EPA/DHA supplementation shifted the cardiac CYP-eicosanoid profile in rats from AA- to EPA- and DHA-derived metabolites. One of these novel metabolites (17,18-epoxyeicosatetraenoic acid; 17,18-EETeTr) exerted a negative chronotropic effect, reduced the ß-adrenergic response and protected against Ca^{2+}-overload in neonatal rat cardiomyocytes (in collaboration with Gerd Wallukat). 17,18-EETeTr was effective with an EC_{50} of 1-2 nM. We concluded that this metabolite may mediate the antiarrhythmic effect of omega-3 fatty acids. We then identified the 11,12-double bond and the 17(R),18(S)-epoxy group as the structural elements essential for the biological activity of 17,18-EETeTr (in collaboration with John R. Falck, UT Southwestern, Dallas). Moreover, we have developed and patented synthetic agonists with improved chemical and biological stabilities as a basis for novel antiarrhythmic drugs.

Role of catechol-o-methyltransferase (COMT) and 20-HETE in acute kidney injury

Acute kidney injury (AKI) is a severe problem in various clinical settings ranging from renal transplantation to cardiac surgery. Collaborating with Duska Dragun (Charité, Berlin), we found that carriers of the low activity COMT allele are at significantly higher risk to develop AKI after cardiac surgery. In a recent joint study, we tested the hypothesis that overproduction of 20-HETE during ischemia/reperfusion (I/R) contributes to AKI using an experimental rat model. We found that compounds that inhibit 20-HETE synthesis or 20-HETE action significantly protected against I/R-induced vascular inflammation, tubular injury and loss of renal function (compare figure). These findings may offer novel therapeutic opportunities for preventing AKI and preserving kidney function during transplantation.

Collaborations Jens Titze

Our group has collaborated closely with Jens Titze (University of Erlangen) for the past half-decade. We determined that salt has a non-osmotic storage compartment and that salt elucidates signals regarding angiogenesis (lymph vessels). The findings imply the importance of an osmosensitive transcription factor (TonEBP) and suggest mechanisms to test the salt-resistant or salt-sensitive hypertension hypothesis. We will rely on sodium-directed magnetic resonance imaging and spectroscopy to test our findings in humans.

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Dominik N. Müller
(Delbrück Fellow)

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Mechanisms of Hypertension-Induced Target Organ Damage

Dominik N. Müller leads a group of young investigators pursuing the question of how hypertension induces target-organ damage. In a translational approach funded by an ECRC grant, Ralf Dechend (Helios Clinic) and Dominik Müller focus primarily on the placenta, heart and kidneys. The primary mediator that has captured their attention is the renin-angiotensin system. The group also cooperates closely with MDC scientists. The group has also been a resource for young clinicians and doctoral students beginning their careers in experimental cardiovascular research.

The group collaborated with Michael Bader and Oliver Daumke on the (pro)renin receptor, with Wolf-Hagen Schunck and Robert Fischer (Charité) on target organ protective role of polyunsaturated fatty acid and the identification eicosanoid receptors, with Ruth Schmidt-Ullrich and Claus Scheidereich on the role of NF-κB in target organ damage, with Martin Zenke (Aachen) to explore the role of the "inhibitor of differentiation 2" (ID2) in hypertension-induced kidney injury, and with Markus Kleinewietfeld to elucidate the protective role of regulatory T cells (Tregs) in hypertension-induced cardiac injury. Finally, the group made the novel observation that the human renin promulgates obesity in transgenic rats by inducing changes in energy metabolism.

Immune mechanisms in hypertension

Mice deficient for Id2(-/-) lack Langerhans and splenic CD8a+ dendritic cells, have reduced natural killer cells, and have altered CD8 T-cell memory. Petra Gratze and the group tested the hypothesis that an alteration in the number and quality of circulating blood cells caused by Id2 deletion would ameliorate angiotensin (Ang) II-induced target-organ damage. Id2-/- mice failed to develop an increase in blood pressure when infused with Ang II and had no target-organ damage. The group conducted kidney (figure 1) and bone marrow transplants that did not restore the sensitivity to Ang II. They also found that vascular smooth muscle cells from Id2-/- mice showed an antisenescence phenotype. The study identified a previously undefined role for Id2 in the pathogenesis of Ang II-induced hypertension. This role is being further explored.

Heda Kvakan led the project studying the role of immunosuppressive CD4+CD25+ regulatory T (Treg) cells in the pathogenesis of hypertensive target organ damage. The group conducted adoptive transfer of Treg cells into Ang II-infused hypertensive mice. Treg cell recipients exhibited improved cardiac hypertrophy and less cardiac fibrosis despite sustained hypertension. Amelioration of cardiac morphology was accompanied by an improvement in arrhythmogenic electric remodeling, indicating the functional significance of the enhanced cardiac morphology. Pronounced connexin 43 immunoreactivity was found at the lateral borders of cardiomyocytes in Ang II-treated mice, implicating this gap-junction protein in the arrhythmias. In contrast, connexin 43 was restricted to the intercalated disk regions in sham controls. Surprisingly, Ang II+Treg-treated mice showed normal connexin 43 gap junction protein localization. Thus, Treg cells ameliorated cardiac damage and accounted for the improved electric remodeling independently of blood pressure-lowering effects. The results provide new insights into the pathogenesis of hypertensive cardiac damage and could therefore lead to new therapeutic approaches that involve manipulation of the immune system.

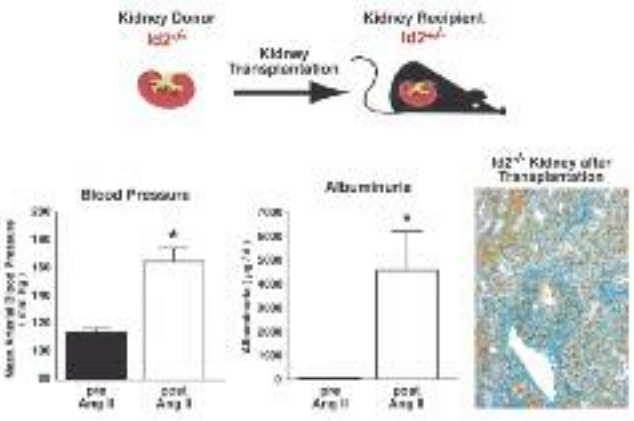
The (pro)renin receptor

The Müller laboratory collaborates with Genevieve Nguyen (INSERM Paris), Michael Bader and Oliver

Daumke (both MDC) on studying the (pro)renin receptor, a transmembrane protein that binds either renin or prorenin. Prorenin is activated by this binding to allow the cleavage of angiotensinogen to Ang I (and then to Ang II through the angiotensin converting enzyme). Independent of that process, the (pro)renin receptor also signals via extracellular-regulated kinase (ERK1/2) and can activate the transforming growth factor (TGF) pathway. Others claim that by blocking the (pro)renin receptor, by means of a decoy peptide sequence termed the handle-region peptide (HRP), the vascular damage caused by diabetes mellitus can be completely ameliorated. The Müller laboratory has worked intensively on this important problem. Sandra Feldt was primarily responsible for this project. The group tested whether human prorenin and renin induce ERK1/2 activation and whether the direct renin inhibitor aliskiren or the HRP inhibits the receptor. The (pro)renin receptor mRNA and protein was detected in isolated human monocytes and in U937 monocytes. In U937 cells, the group found that both human renin and prorenin induced a long-lasting ERK1/2 phosphorylation despite Ang II type 1 and 2 receptor blockade. A mitogen-activated protein kinase kinase 1/2 inhibitor inhibited both renin and prorenin-induced ERK 1/2 phosphorylation. Neither aliskiren nor HRP inhibited binding of (125)I-renin or (125)I-prorenin to the (pro)renin receptor. Fluorescence-activated cell sorter analysis showed that, although fluorescein isothiocyanate-labeled HRP bound to U937 cells, HRP did not inhibit renin or prorenin-induced ERK1/2 activation. Thus, prorenin and renin-induced ERK 1/2 activation are independent of Ang II. The signal transduction is different from that evoked by Ang II. Aliskiren has no (pro)renin receptor blocking effect and did not inhibit ERK1/2 phosphorylation or kinase activity. There was no evidence that HRP affects renin or prorenin binding and signaling. The Council for High Blood Pressure Research of the American Heart Association voted this work as the "best basic paper of the year", published in the journal *Hypertension*.

Renin and metabolism

Renin initiates Ang II formation and (aside from the (pro)renin receptor) has no other known functions. Petra Gratze and the Muller group observed that transgenic rats (TGR) overexpressing the human renin gene (hREN) developed moderate obesity with increased body fat mass and glucose intolerance compared with nontransgenic Sprague-Dawley (SD) rats. The metabolic changes were not reversed by an angiotensin-converting enzyme inhibitor, a direct renin inhibitor, or by (pro)renin receptor blocker treatment. The obese phenotype in TGR(hREN) originated from higher food intake, which was partly compensated by increases in resting energy expenditure, total thermogenesis (postprandial and exercise activity), and lipid oxidation during the first 8 weeks of life. Once established, the difference in body weight between TGR(hREN) and SD rats remained constant over time. The group observed no changes in the cocaine and amphetamine-regulated transcript, pro-opiomelanocortin, both anorexigenic, or neuropeptide Y, orexigenic, mRNA levels in TGR(hREN) versus SD controls. However, the mRNA level of the agouti-related peptide, orexigenic, was significantly reduced in TGR(hREN) versus SD controls at the end of the study, which indicates a compensatory mechanism. The group suggested that the human renin transgene initiates a process leading to increased and early appetite, obesity, and metabolic changes not related to Ang II. The mechanisms are independent of any currently known renin-related effects. The novelty here is the introduction of renin as an obesity-related enzyme. The group is busy exploring mechanisms further.



Renal Id2 deficiency does not the genesis of hypertension and renal damage.

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Rainer Dietz

Structure of the Group

Group Leader
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Dr. Stefan Donath
Dr. Jens Fielitz
Dr. Robert Fischer
Dr. Jan Monti
PD Dr. Cemil Özcelik
Dr. Laura Zelarayán

Clinical Cardiology

The main topic of the group is to address the molecular signaling pathways leading to heart failure. Heart failure, a highly heterogenous disease, is characterized by a symptom complex of congestion and exertional fatigue. Heart failure is mainly caused by coronary artery disease/myocardial infarction, arterial hypertension, dilated and hypertrophic cardiomyopathies, and inflammatory heart disease. In a translational approach new animal models have been established mimicking those human cardiovascular disorders and their effects. Detailed phenotyping of those heart failure models has revealed new insights into disease development and adaptive compensatory pathways prior to the onset of heart failure which may lead to new preventive and therapeutic strategies.

Background

Although great progress has been achieved in the treatment of heart failure, it remains a leading cause of death and disability throughout the world and prevalence and incidence increase year-by-year. Heart homeostasis is regulated by apoptosis, cardiomyocyte hypertrophy and myocardial regeneration determining the hearts ability to response to pathologic loads. The balance between cardiomyoycte survival and apoptotic pathways as well as heart regeneration versus deleterious cardiac remodelling appear to be key determinants of the transition from heart hypertrophy to ventricular dilatation.

Complex Genetics of Hypertension and Heart Failure

Jan Monti, Jens Buttgereit

In humans, hypertension associated with sustained cardiac hypertrophy represents one of the most common causes of heart failure. Starting from high blood pressure, the pathophysiological cardiac remodelling cascade proceeds to left ventricular hypertrophy as a primarily adaptive process. Later on, left ventricular dilatation, decreased systolic function, and cardiac arrhythmias can occur in some patients, whereas others retain stable systolic function without clinical signs of heart failure.

Heart failure is thought to result from complex interactions between genetic susceptibility and life style/environmental factors. In close collaboration with Norbert Hübner, Jan Monti aimed to identify gene variants underlying heart failure in the spontaneously hypertensive heart failure (SHHF) rat. They generated experimental evidence that genetic variance in the Ephx2 (soluble epoxide hydrolase) gene facilitates progression from hypertension and cardiac hypertrophy to heart failure in that rat model. The demonstrated causative impact for Ephx2 in the complex initiation of heart failure in rats and mice inaugurate Ephx2 as a new heart failure treatment avenue.

Hypertension and Heart Failure

Ralf Dechend, Florian Herse, Katrin Wenzel

In addition to genetic predisposition the subgroup of Ralf Dechend examined the relevance of “agonistic” autoantibodies directed against the α1-adrenergic receptor in patients with refractory hypertension. Almost half of them displayed α1-autoantibodies. Interestingly, the removal of the α1-autoantibodies by immunoabsorption significantly reduced blood pressure during the observed follow-up phase of 180 days. By this innovative therapeutic approach Ralf Dechend

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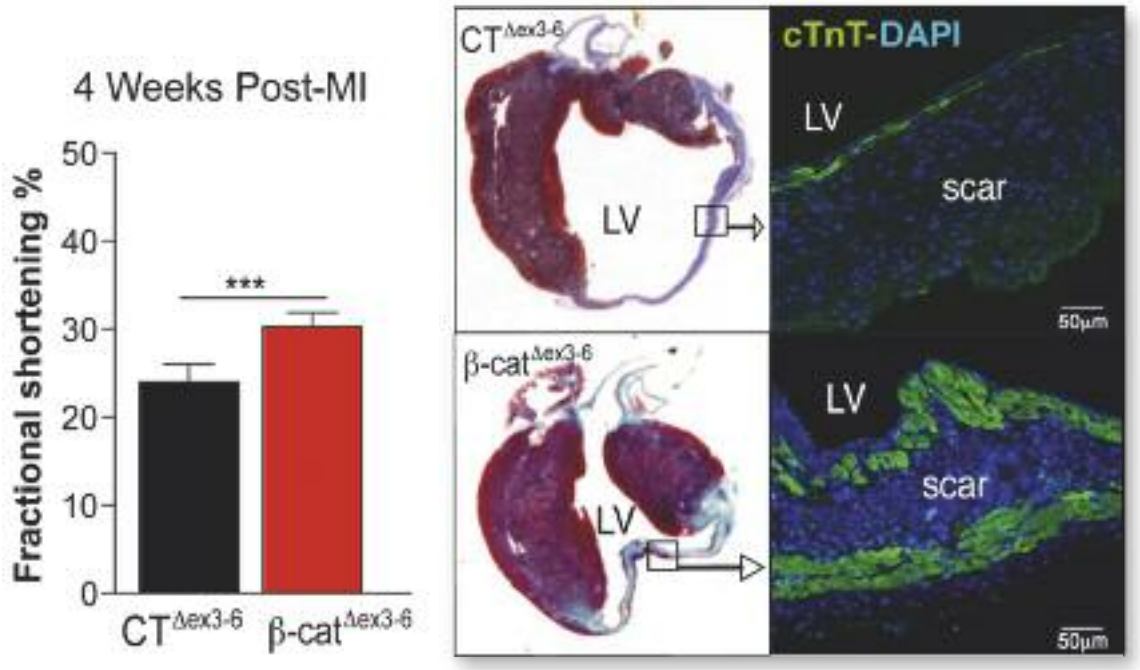

Figure 1. *αMHC-dependent β-catenin depletion attenuates post-infarct LV remodeling. β-catenin depletion (β-cat $^{\Delta ex3-6}$) results in improved fractional shortening 4 weeks after infarct associated with a prominent sub-endocardial and sub-epicardial layer of cardiomyocytes as identified by Troponin T (cTnT) staining.*

and his colleagues provide evidence that α1-autoantibodies are of potential pathophysiological relevance and could represent a factor contributing to the development of refractory hypertension.

Experimental Electrophysiology in Heart Failure

Robert Fischer

In endstage heart failure a significant portion of heart failure patients die suddenly due to malignant arrhythmias. Robert Fischer and his subgroup are performing electrophysiological examinations in murine models to evaluate the risk of sustained ventricular arrhythmias. Recently, they were able to substantiate the beneficial effects of omega-3-polyunsaturated fatty acids in preventing arrhythmias in a heart failure model.

Patent Application 01/2009: EP18174 "Novel eicosanoid derivatives".

Genetics of Hypertrophy and Heart Failure

Cemil Özcelik, Christian Geier, Andreas Perrot, Maximilian Posch

Hypertrophic cardiomyopathy (HCM) is the most common genetic myocardial disease with a prevalence of 0.2 % in adults. Left ventricular hypertrophy in the absence of other causes is the clinical hallmark. In addition, the clinical phenotype is characterized by sudden cardiac death. Mutations in a number of sarcomeric contractile-protein genes are causative in approximately 60 % of individuals with HCM. Christian Geier from Cemil Özcelik´s subgroup has identified a missense

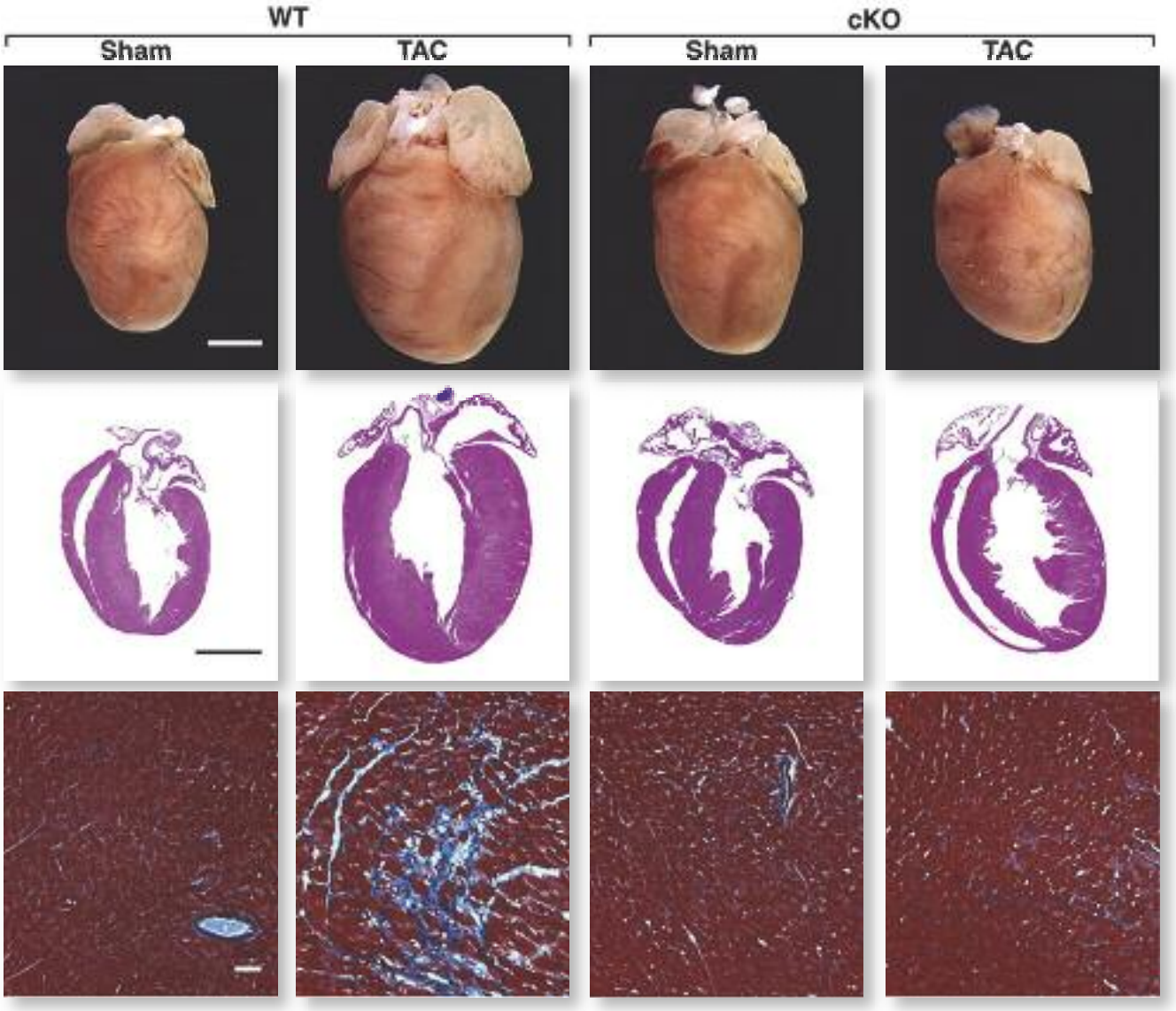

Figure 2. *Cardiac-restricted ablation of PKD1. Upon the onset of pressure overload PKD1 mutants showed less hypertrophy and a significant reduction in cardiac fibrosis. TAC: Thoracic aortic banding.*

mutation in the "muscle LIM protein" (MLP) in patients with HCM. Surprisingly, immunohistochemical analysis revealed that MLP is mainly localized in the cytosolic component, rather than in the sarcomere, indicating that HCM is not exclusively a sarcomeric disease. Furthermore, their data suggested that impaired mechano-sensory stress signaling might be involved in the pathogenesis of HCM.

Nuclear Receptors in Cardiac Metabolism

Florian Blaschke

The cardiac phenotype of left ventricular hypertrophy caused by genes that are involved in metabolism and energy production underscores the impact of energetic pathways on cardiac phenotype and function. A better understanding of the role of cardiac energy metabolism and hypertrophic gene expression may consequently lead to more effective therapeutic strategies for

the prevention and treatment. The subgroup of Florian Blaschke is elucidating the role of those nuclear receptors in cardiac metabolism and hypertrophy both in vitro and in vivo and characterize the molecular mechanisms utilized to regulate gene expression involved in cardiomyocyte growth and energy metabolism.

Apoptosis and Regeneration in Heart Failure

Stefan Donath, Junfeng An

Another focus of the group has addressed the issue of apoptosis in heart failure progression. ARC (apoptosis repressor with caspase recruitment domain) is a master regulator of cardiac death signaling, as it is the only known factor that specifically inhibits both death receptor and mitochondrial apoptotic death pathways. By generating ARC deficient mice the subgroup of Stefan Donath attempted to elucidate the physiological role of ARC in the heart. Upon biomechanical stress induced by aortic banding, ARC null mice developed accelerated cardiomyopathy compared with littermate controls that was characterized by reduced contractile function, cardiac enlargement, and myocardial fibrosis. Likewise, ischemia-reperfusion injury of ARC null mice resulted in markedly increased myocardial infarct sizes. The pathophysiological relevance was underscored by specimens from failing human hearts showing markedly reduced ARC protein levels. Thus, they identified a tissue-specific anti-apoptotic factor which is downregulated in human failing myocardium and which is required for cardioprotection in pressure overload and ischemia. Inhibition of apoptosis might have a similar effect on restoration of the balance between loss of cardiomyocytes and heart renewal by strengthening the self-renewal properties of the heart.

Transcription Factors in Myocardial Stress Remodelling

Laura Zelarayán, Martin Bergmann (guest scientist), Maria-Patapia Zafiriou

The subgroup of Laura Zelarayán and Martin Bergmann elucidated the role of the Wnt/β-catenin pathway in activation of endogenous cardiac progenitor cells. Therefore, they deleted the β-catenin gene in the myocardium by using the Cre/loxP technology. The conditional knockout of β-catenin resulted in cardiac hypertrophy, suggesting that β-catenin downregulation might be part of the adaptive cardiac hypertrophy response. Additionally, the depletion of β-catenin positively affected LV-function and survival after experimental myocardial infarction (figure 1). Furthermore, conditional β-catenin deficient mice displayed better remodelling upon cardiac stress. Isolated Sca-1^{pos} cells from mutant mice showed significantly increased differentiation capacity.

Pathological Cardiac Remodelling in Heart Failure

Jens Fielitz

A further gene family that suppresses stress-dependent remodelling of the heart via association with the MEF2 transcription factor are class II histone deacetylases (HDACs). Protein kinase D (PKD) is a stress-responsive kinase that phosphorylates class II HDACs, resulting in their dissociation from MEF2 with consequent activation of MEF2 target genes. The subgroup of Jens Fielitz was able to show that the cardiac ablation of PKD1 showed less hypertrophy and a dramatic reduction in cardiac fibrosis compared to wild type mice when subjected to aortic banding (figure 2). In addition, PKD1 mutant mice were resistant to left ventricular dilatation and a decrease in contractility. This study indicates that PKD1 functions as a key transducer of stress stimuli involved in pathological cardiac remodelling in vivo. These findings provide the first evidence that deletion of a class II HDAC kinase in vivo diminishes stress-induced hypertrophy.

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Dr. Sabine Klaassen
Dr. Bertold Struk

Cardiovascular Molecular Genetics

Molecular genetics of cardiomyopathies, regeneration of the embryonic heart and elucidation of the molecular pathology/genetics of vascular lesions are the major topics of our research group. A number of cardiomyopathy causing mutations have been identified by our research group and the molecular pathology of those mutations is currently under investigation. The capacity of the embryonic heart to regenerate from genetic damage is based on a complex mechanism of compensatory proliferation, inhibition of apoptosis, and adaptation of various signaling pathways. Liver X receptor agonists are potential targets for the treatment of metabolic, inflammatory and cardiovascular diseases and negatively interfere with cytokine-induced nuclear receptor corepressor dissociation from the C-reactive protein promoter, thus maintaining this gene in a repressed state.

Genetics of cardiomyopathies

Brenda Gerull, Sabine Klaassen, Arnd Heuser, Michael Gramlich

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited heart disease predominantly affecting the right ventricle and a prevalent cause of ventricular arrhythmias and sudden death, especially in young adults. Genetically, heterozygous loss-of-function mutations in desmosomal proteins (desmocollin2, desmoglein2, plakoglobin, desmoplakin, and plakophilin2) have been mainly associated with ARVC. We have described 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 (see figure 1) known to be essential for maintaining tissue integrity and increasingly implicated in cell signaling. While the involvement of multiple desmosomal protein genes has led to speculation regarding the sensitivity of myocardium to mechanical disruption, the pathogenic mechanisms leading to ARVC in humans are largely unknown. We currently try to elucidate the genetic and molecular mechanisms of various human PKP2 mutations and their consequences in the pathology of the intercellular junction complex using cell culture experiments and transgenic mouse models. Furthermore, we investigate different knockout models of desmosomal components to define the adhesive and signaling contributions of thesel proteins in the maintenance of cardiac tissue.

Left ventricular noncompaction of the myocardium (LVNC) has recently been recognized as a distinct primary cardiomyopathy with a genetic etiology. LVNC is characterized by a unique congenital cardiac morphology, consisting of numerous, excessively prominent ventricular trabeculations and deep intertrabecular recesses. The heterogeneity of the clinical features includes progressive deterioration in cardiac function resulting in congestive heart failure, arrhythmias, thrombembolic events, and sudden cardiac death. The disorder is assumed to result from an intrauterine arrest in the process of compaction of the developing myocardium. We found mutations in genes encoding sarcomere proteins in a significant proportion of LVNC patients. Heterozygous mutations in genes encoding – myosin heavy chain (*MYH7*), -cardiac actin (*ACTC*), and cardiac troponin T (*TNNT2*) account for 17% of cases of isolated LVNC in adult patients. As hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy

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(DCM) are also caused by mutations in sarcomere protein genes our analyses support the concept of a shared molecular etiology of these different cardiomyopathic phenotypes.

Mutations in the giant sarcomeric protein *TTN* are responsible for inherited dilated cardiomyopathy (DCM) in humans. To dissect affected pathways leading to titin-based DCM and to investigate the role of titin in cardiac development, we generated a mouse model that mimics a *TTN* truncation mutation (c.43628insAT) previously identified in a large family with autosomal dominant DCM. Heterozygous mice chronically exposed to cardiac stress displayed features of DCM, thereby recapitulating the human phenotype. The mutant embryos homozygous for the *Ttn* knock-in mutation showed severe defects in sarcomere formation and died before E9.5 due to non-beating hearts. Our data demonstrate that the removal of titin's M-line region leads to unformed sarcomere structures and provide a chronological relationship between titin expression, sarcomere formation, and embryonic lethality due to absent cardiac contractile function.

Regeneration of the embryonic heart

Jörg Drenckhahn

Regeneration of functional myocardium after cardiac injury is currently one of the most rapidly developing fields in cardiovascular research. Several different approaches based on injection of various cell types as well as mobilisation or stimulation of endogenous cells to repair the damaged heart have been reported. Despite lots of encouraging findings some controversial results have highlighted the need for a better under-

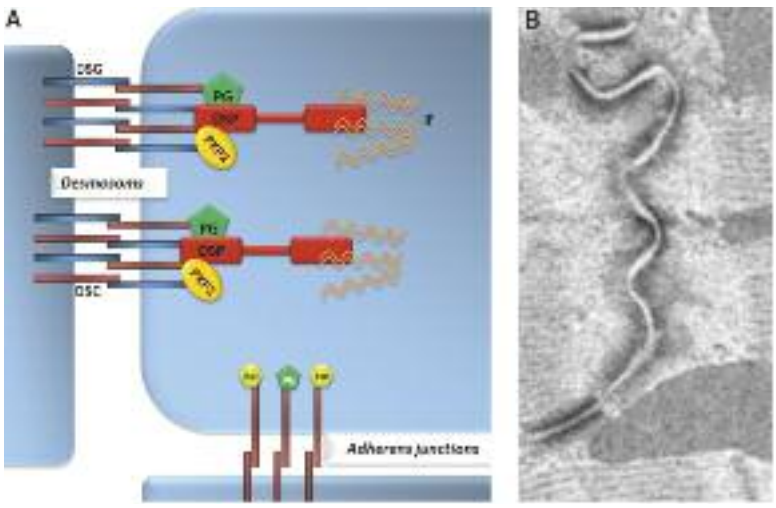

Figure 1. *The structural organization of desmosomes. (**A**) The schematic drawing shows desmosomal cadherins (DSG, desmoglein; DSC, desmocollin); the armadillo-family members plakoglobin (PG) and the plakophilins (PKP); and the intermediate filament (IF)-binding protein desmoplakin (DSP). (**B**) Electron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

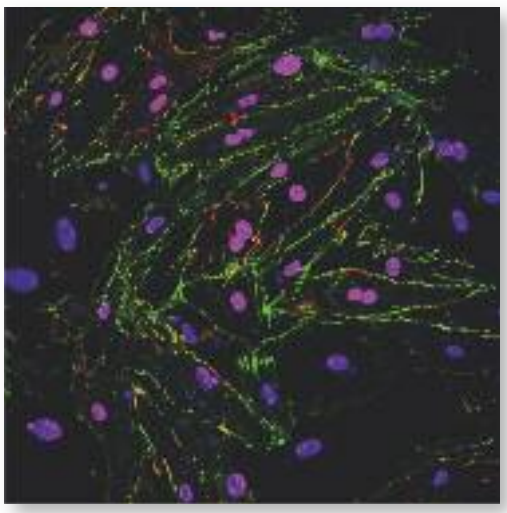

Figure 2. *Immunolocalisation of desmosomal proteins Plakophilin2 (green) and Plakoglobin (red) in neonatal rat cardiomyocytes ; Nkx2.5 (magenta).*

standing of the molecular and cellular mechanisms underlying a solid myocardial regeneration. In this regard, the embryonic heart is a perfect model to study these processes as key events like cardiomyocyte proliferation, differentiation as well as regional and functional specification occur physiologically in the developing heart.

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* knock-out (KO) males, heterozygous females appeared normal during the first months of life with surprisingly few clusters of affected cardiomyocytes, considering an expected mosaic of affected and normal cardiomyocytes as a result of random X chromosomal inactivation. However, analyses of heterozygous female 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 heterozygous *Hccs* KO females do not appear completely normal but show morphological, cellular as well as molecular signs of immaturity. These changes, however, normalize until adulthood suggesting activation of compensatory cardiac growth mechanisms in the postnatal heart after disturbed heart development.

The detailed characterisation of molecular signaling pathways as well as cell types involved in embryonic heart regeneration is currently underway and should provide major new insights into heart development and cardiac organ size control. Furthermore, the identification of regenerative factors and stimuli within the embryonic heart might potentially enable the development of new therapeutic strategies for cardiac repair in the adult. Finally, this model might provide a useful tool to study the impact of disturbed heart development on the incidence of postnatal cardiac disease in the context of fetal programming.

Nuclear Receptors as Potential Target for the Treatment and Prevention of Metabolic, Inflammatory and Cardiovascular Disease

Florian Blaschke

Members of the nuclear receptor superfamily of ligand-dependent transcription factors play essential roles in development, homeostasis, reproduction and immune function. Several members of this family, including the estrogen receptor (ER) and peroxisome proliferator-acti-

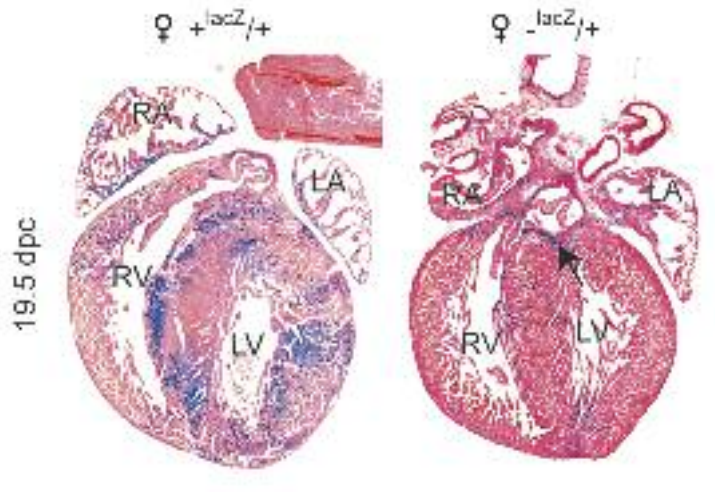

Figure 3. *β-Galactosidase staining of 19.5 dpc (days post coitum) fetal hearts prior to birth (counterstained with Eosin). The control heart (♀+lacZ/+, heterozygous for an X-linked lacZ reporter gene) shows large patches of β-Gal positive and negative cells due to random X chromosome inactivation. In the heterozygous Hccs-knock-out heart (♀-lacZ/+) the lacZ reporter gene is linked to the X chromosome carrying the defective Hccs gene allowing the detection of Hccs deficient cells by β-Gal staining. Due to cardiac regeneration during embryonic development the proportion of Hccs deficient cells is minimized until birth. Very few isolated Hccs deficient cardiomyocytes can be detected within the ventricular and atrial myocardium with only the basal region of the interventricular septum showing a larger cluster of β-Gal positive cells (see arrow). RA = right atrium, LA = left atrium, RV = right ventricle, LV = left ventricle.*

vated receptors (PPARs) are target of drugs that are used in a variety of clinical settings. The ability of nuclear receptors to switch from a transcriptional repressor to a transcriptional activator by binding of synthetic or natural ligands provided important insight into the mechanism(s) of gene regulation. Given their pleiotropic effects and their activation by specific ligands, new drugs targeting nuclear receptors are emerging as promising therapeutics for the treatment of cardiovascular, metabolic and inflammatory disease.

Activation and proliferation of vascular smooth muscle cells (VSMCs) are recognized to play a decisive role in vascular proliferative diseases such as primary atherosclerosis, postangioplasty restenosis, vein graft disease, and transplant vasculopathy. Due to the crucial role of VSMCs in the pathobiology of vascular occlusive disease, a pharmacologic blockade of VSMC activation and the cell cycle machinery is a promising therapeutic approach to prevent and treat vascular proliferative disease. Using both *in vitro* and *in vivo* approaches, we are investigating the role of various nuclear receptors and their activation by synthetic ligands in VSMC activation and cell cycle progression and are characterizing the molecular pathways utilized to regulate gene expression.

Cardiac hypertrophy leading to heart failure is a major cause of morbidity and mortality worldwide. The reasons why cardiac dilatation and failure eventually occur are unknown although alterations in energy status and metabolism are proposed to play an important role. We are elucidating the role of nuclear receptors in cardiac metabolism and hypertrophy both *in vitro* and *in vivo* and characterize the molecular mechanism(s) utilized to regulate gene expression involved in cardiomyocyte growth and energy metabolism.

Molecular Genetics of Pseudoxanthoma elasticum (PXE)

Berthold Struk

Pseudoxanthoma elasticum is a heritable systemic disorder of the elastic tissue characterized by degenerative calcification with subsequent disintegration and destruction of the elastic tissue of several organs. Cardiovascular disease encompasses a wide clinical spectrum from mental fatigue syndrome to early cardiovascular death due to myocardial infarction or, very rarely, gastrointestinal hemorrhage. We have previously mapped the PXE locus to a 500 kb interval on chromosome 16p13.1. and have shown that mutations in a transmembrane transporter protein, ABC-C6 (also known as MRP-6), cause PXE. Recently, an extensive mutation screen of 81 PXE families revealed 59 distinct ABC-C6 mutations. The mode of inheritance in this cohort is solely recessive or pseudo-dominant if a diseased individual married a carrier. Our data do not support any evidence for a dominant mode of inheritance of PXE. Current research focuses on the identification of molecular mechanisms for disease development in the different tissues involved.

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Magnetic Resonance

The group's 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 of the benefits and challenges of ultrahigh-field imaging to advance cardiovascular, neurovascular, molecular and other MRI applications. These efforts are designed to spatially resolve and characterize (patho)physiological processes and biophysical mechanisms to promote a transfer from basic research to (pre)clinical studies and vice versa. However, signal-to-noise ratio (SNR) and imaging speed have become an increasingly stringent limit in new MRI applications. Promising in this regard is the increase in magnetic field strengths available for both animal (9.4 T) and whole-body MR (7.0 T) scanners, though ultrahigh-field MRI has earned the moniker of being among the most challenging MRI applications.

Myocardial T_2* Mapping

Emerging cardiovascular MRI applications include T_2* relaxation sensitized techniques which are increasingly used in basic research and (pre)-clinical imaging. For these reasons, an imaging strategy that avoids image distortions and has the advantage that susceptibility weighting can be adjusted from zero upwards is an appealing strategy in anatomically accurate T_2* mapping of the heart. Members of our group patented and established a free-breathing, cardiac-gated, susceptibility weighted fast spin-echo technique in conjunction with black blood preparation and navigator gated respiratory motion compensation. The applicability of this approach has been demonstrated in myocardial T_2* imaging/mapping (Figure 1) at 3.0 T and is now being transferred to the 7.0 T whole body scanner and the 9.4 T animal system.

Development of Novel Synchronization Techniques

Obtaining MRI images of moving organs requires speed and efficiency due to physiological motion and flow constraints, which dictate the viable window for data acquisition. Cardiac motion is commonly dealt with using electrocardiographic (ECG) gating techniques to synchronize data acquisition with the cardiac cycle. As ultrahigh-field cardiac MRI becomes more widespread, the sensitivity of ECG recordings to interference from electromagnetic fields and to magneto-hydrodynamic effects increases, requiring a practical gating/triggering alternative. For these reasons, an MR-stethoscope has been proposed and clinically evaluated by members of our group to successfully meet the demands of cardiac triggered MRI at 7.0 T (Figure 2).

Rapid Functional Brain Mapping Free of Image Distortion

The potential of higher magnetic field strengths for functional brain mapping in clinical practice and basic research has yet to be fully realized. Here, one important question is the choice of pulse sequence for optimal image quality in functional brain imaging. Echo Planar Imaging (EPI) is the most frequently applied technique for fMRI because images may be acquired rapidly and they are inherently sensitive to Blood Oxygen Level Dependent contrast. However, a significant drawback of EPI is its sensitivity to inhomogeneities in B_0, leading to signal loss and image distortion in regions of spatially varying magnetic susceptibility. These effects are more apparent at high and ultra-

Christof Thalhammer (as of 11/09)

Secretariat
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Start of Group: August 2009

high magnetic field strengths B_0. Rapid spin echo based pulse sequences successfully developed by members of our group offer a distortion-free alternative to EPI for fMRI. Consequently, activation may be mapped throughout the brain using spin-echo based methods with greater confidence than when using EPI. The challenges to spin echo techniques are adequate temporal resolution for fMRI and avoiding excessive RF power deposition which will be a focus in the anticipated research program.

RF Coil Developments

The sensitivity advantage afforded by ultrahigh-field imaging is the driving force behind several technological developments. One ongoing development pioneered by our group is a broad move towards MR systems with 32 or more receiver channels. This includes the development and clinical evaluation of many element RF coil arrays designed for RF transmission and signal reception. Here, the group's research activities aim to go beyond conventional proton imaging to foster explorations into imaging of sodium, fluor, and other nuclei to gain a better insight into metabolic and molecular processes.

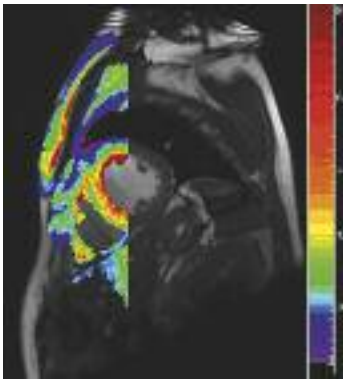

Figure 1. *Quantitative T_2*-relaxation map of the human heart superimposed to a short axis anatomical view of the human heart. The myocardial T_2*-mapping approach proposed by our group is of value for the assessment of myocardial iron content and myocardial tissue oxygenation.*

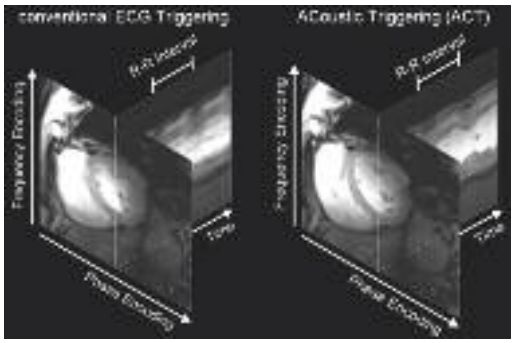

Figure 2. *Short axis views of the heart together with whole R-R interval time series of one-dimensional projections along the dotted line profile marked in the short axis view. CINE images were obtained at 7.0 T using conventional ECG (left) the ACoustic Triggering (ACT) (right) approach proposed by our group. ECG triggered CINE imaging was prone to severe cardiac motion artifacts due to R-wave misregistration. In comparison, acoustically triggered CINE imaging at 7.0 T produces images of the human heart free of motion artifacts.*

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T. Frauenrath, F. Hezel, U. Heinrichs, S. Kozerke, J. F. Utting, M. Kob, C. Butenweg, P. Boesiger, T. Niendorf (2009) Feasibility of Cardiac Gating Free of Interference with Electro-Magnetic Fields at 1.5 Tesla, 3.0 Tesla and 7.0 Tesla Using an MR-Stethoscope. Invest. Radiol., [Epub ahead of print].

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Patent applications

T. Niendorf, U. Heinrichs
Method for Susceptibility Weighted, Distortion Free Magnetic Resonance Imaging of the Cardiovascular System
Patent DE 10 2007 045 172.7 (2008)

T. Niendorf, F. Hezel
Method for Automatic Motion Analysis and Quality Assurance in Cardiovascular Magnetic Resonance Imaging
Patent application 10 2009 011 382.7 (2009)

Michael Bader

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Cardiovascular Hormones

The group focuses on the molecular biology and function of hormone systems involved in cardiovascular regulation. The physiological functions of these systems are analyzed 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, aiming to apply these fields to the rat and to establish gene targeting in this species.

Renin-angiotensin system

Natalia Alenina, Brit Rentzsch, Mihail Todiras, Ping Xu, Aline Hilzendeger, Luiza Rabelo, Sergio Santos, Philip Boyé, Gabin Sihn, Cibele Cardoso

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 renin receptor, angiotensin(1-7) and its receptor Mas, have elucidated the physiological function of these factors. Together with transgenic rats overexpressing this peptide, Mas-knockout mice characterized the angiotensin(1-7)/Mas system as a cardioprotective axis that counteracts the classical RAS effects in particular improving endothelial function. Furthermore, these animals showed that angiotensin (1-7) and Mas are important for insulin sensitivity and the pathogenesis of metabolic syndrome.

Kallikrein-kinin system

Ines Schadock, Robert Fischer, Marcelo Mori, Carlos Barros, Alessander Guimaraes, Fatimunnisa Qadri, Anthony Rousselle, Johan Duchene, Vanessa Merino

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 involved in 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 basic regulation of the cardiovascular system but is involved in the pathogenesis of multiple diseases and, thus, a relevant drug target.

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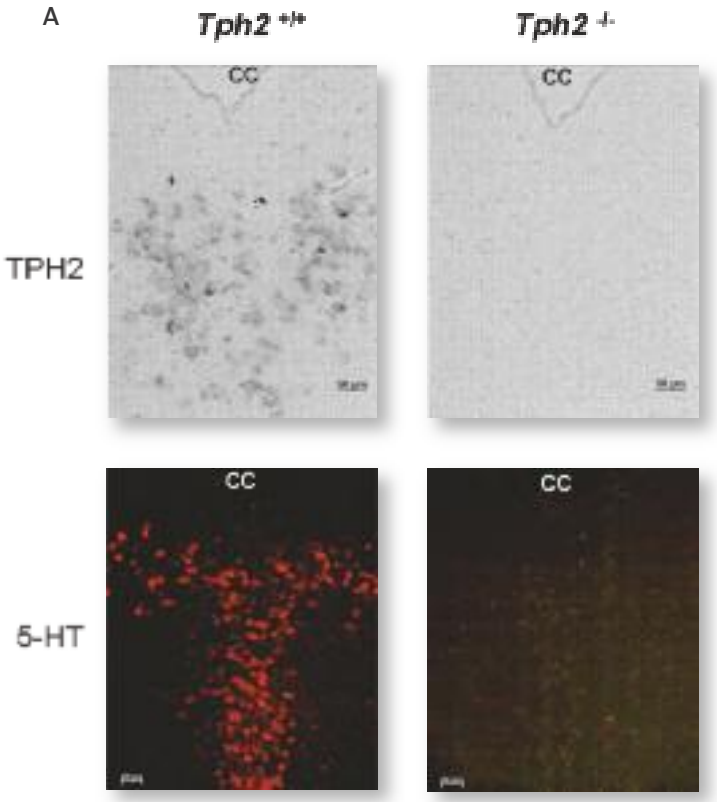

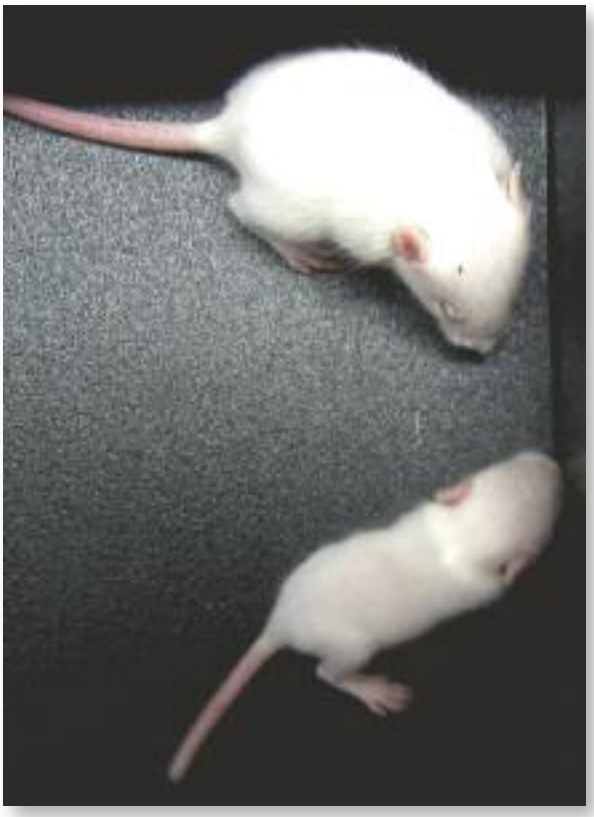

*Growth retardation in Tph2-deficient mice lacking serotonin in the brain. (**A**) Absence of Tph2-mRNA and serotonin (5-HT) immunoreactivity in the brain stem of Tph2-deficient mice. (**B**) A 15 days old Tph2-deficient mouse (below) is much smaller than a wild-type littermate (above).*

Natriuretic peptide system

Jens Buttgereit

There are 3 natriuretic peptides (NP), ANP, BNP, and CNP, which interact with two natriuretic peptide receptors, NPR-A and NPR-B, to induce a multitude of actions in heart, kidney, vessels, brain and other tissues. The receptors are dimeric molecules, which after activation synthesize cyclic GMP. We have shown that dimerization is essential for the activation of the receptors and have designed dominant negative mutants to downregulate the activity of the receptors in cells and transgenic animals. Transgenic rat models expressing a dominant negative mutant for NPR-B exhibit sympathetic activation and develop cardiac hypertrophy supporting a cardioprotective action of this receptor and its ligand CNP. Moreover, these animals show an impaired bone growth in accordance with the phenotype of knockout mice for NPR-B and CNP and humans with mutations in the NPR-B gene.

Serotonin system

Natalia Alenina, Dana Kikic, Katja Tenner, Katarina Kotnik, Saleh Bashammakh, Valentina Mosienko, Susann Matthes

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 tryptophan hydroxylases, the rate limiting enzymes 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. 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, and cardiovascular parameters. In addition, these mice exhibit increased aggression and maternal neglect. Furthermore, searching for molecules, which are crucial for the development of serotonergic neurons, we developed protocols for the differentiation of embryonic stem (ES) cells into serotonin-producing neurons. Based on genetic modifications of the ES cells, the method allows to select this neuronal population and to monitor their development in *in-vitro* and *in-vivo* studies.

Androgen receptor

Gabin Sihn, Silke Mühlstedt

In a collaborative work with the Charité studying gender effects in cardiac hypertrophy and failure, the group generates and characterizes animal models with altered androgen receptor expression in distinct cell types of the heart.

Importins

Franziska Rother, Tanja Shmidt, Stefanie Hügel

Importins are essential components of the machinery that transports proteins into the nucleus of eukaryotic cells. In an approach to study the physiological function of alpha importins we have generated knockout mice for all five known paralogs in the mouse. The most obvious phenotype was discovered in mice lacking importin alpha7: Both sexes of these animals are infertile. The molecular basis of this phenotype is currently analyzed. In addition, we could show that the absence of importin alpha5 during mouse development does not significantly interfere with neuronal differentiation and proper brain development, in contrast to the prediction based on a study in cell culture. Comparative studies on alpha-importin deficient mice and cells derived from them will allow to discover novel redundancies and specificities in nuclear transport.

Transgenic and stem cell technology

Alexander Krivokharchenko, Elena Popova, Irina Lapidus, Natalia Alenina, Katarina Kotnik, Larissa Vilianovitch, Ilya Chuykin

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 behavioral studies. However so far, there is no reliable method of generating targeted genetic alterations in these species. In order to obtain rat pluripotent stem cells two methodologies were applied in our group: isolation of ES (embryonic stem) 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 signaling 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 carry-

ing constructs, which express small interference RNAs suited to downregulate specific genes. The first target gene was the insulin receptor yielding a unique inducible rat model for diabetes mellitus type 2.

Selected Publications

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European Young Investigator (EURYI) Award
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Mobile DNA

Transposons ("jumping genes") are discrete segments of DNA that have the distinctive ability to move and replicate within genomes across the tree of life. Transposons offer a new model to study DNA recombination in higher organism, as well as host-parasite interaction. Transposons are also natural gene delivery vehicles that are being developed as genetic tools. Our laboratory is following the strategy of understanding the mechanism of transposition and its regulation and translate this knowledge to derive transposon-based genetic tools for genome manipulation or for gene therapy.

Quality controls in Sleeping Beauty element (SB) transposition

Diana Pryputniewicz, Tobias Jursch, Andrea Schorn

Our understanding the way 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, at which certain molecular requirements have to be fulfilled for the transpositional reaction to proceed. The role of these regulatory checkpoints is to avoid accumulation of incorrect reaction products in genomes, possessing a threat of genome instability associated by transposition. We also investigate the possible involvement of small, germline-specific RNAs in transposon regulation in zebrafish, as well as the effect of chromatin structure of both donor and target sites on transposition.

Transposon-host interactions

Yongming Wang, David Grzela, Anantharam Deveraj, Andrea Schmitt

Transposons occupy a significant portion of our genomes. However, 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 in the genome and are able to sense developmental and environmental changes and manipulate stress signaling. Cellular mechanisms that are directly involved in repairing transposition-inflicted DNA lesions or can attenuate DNA damage should have crucial role in establishing stable host-transposon co-existence. Our results suggest that SB transposon takes advantage of the cellular repair machinery and/or during DNA replication to amplify their own genome.

Domesticated, transposon-derived cellular genes

Csaba Miskey, Marta Swierczek

One particular copy of the transposase gene of the ancient *Hsmar1* human transposon has been under selection. This transposase coding region is part of the *SETMAR* gene, in which a histone methylatransferase SET domain is fused to an Hsmar1 transposase domain. SETMAR retains ist ability to bind to transposon sequences *in vitro*, and we are currently investigating the cellular function of this gene by integrated genomic and transcriptomic approaches.

The active copy of a rat endogenous retrovirus

Yongming Wang

Endogenous retroviruses were repeatedly demonstrated to influence the expression of rat genes, without knowing the active copy. A phenotype of hypodactyly in rat was associated with recent retroviral activity. We

have identified an "active" copy of novel endogenous retrovirus in the rat genome, and showed that the element is active in retrotransposition. The transpositionally active copy has an intact *env* gene, so element might be capable of infection.

Genome manipulation – Transposon mutagenesis in rat spermatogonial stem cells

Lajos Mátés, Ivana Grabundzija

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 (transgenesis, genomic screens) in organisms where this technology was not available before. One particular application relates to loss-of-function insertional mutagenesis in rats with the goal of generating knockout animals. 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. The project has enormous potential to develop powerful genomic tools for rat that is the preferred model organism of cardiovascular, as well as toxicology and behavioral studies.

Deciphering the genetic background of neuroblastoma and hormone-induced breast cancer

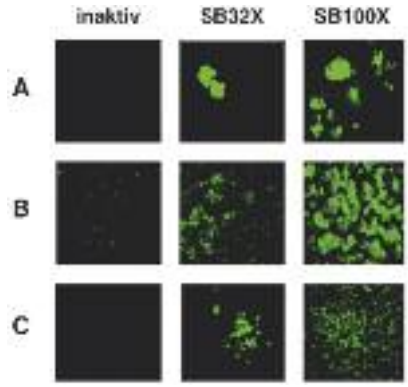
Sanum Bashir

The SB transposon is suitable for somatic mutagenesis and emerged as a new tool in cancer research as an alternative to retroviral mutagenesis. Transposon-based insertional mutagenesis screens are able to identifiy both oncogenes and tumor-suppressor genes. We are engaged in two projects aiming at the discovery of novel driver mutations that are associated with neuroblastoma (in mice) and estrogen-induced mammary cancer (in rats). The transposon mutagenesis approach is expected to be a powerful tool to decipher gene regulatory networks cooperating in cancer development, progression and metastasis.

Transposons as non-viral vectors for gene therapeutic approaches

Ismahen Ammar, Csaba Miskey, Katrin Voigt, Jichang Wang, Frank Schulze

DNA-based transposons are natural gene delivery vehicles, and molecular reconstruction of SB represents a cornerstone in applying transposition-mediated gene delivery in vertebrate species, including humans. Our recently developed 100-fold hyperactive SB system



Stable gene transfer and expression of green fluorescent protein (GFP) in differentiating, human hematopoietic stem cell lineages (A-C). The cells only appear green if the GFP marker gene stably integrated in their chromosomes by transposition. The hyperactive transposase (SB100X) generates far more green cells than an earlier version of the SB transposase (SB32X).

opened new avenues for gene therapeutic approaches, and we are currently developing preclinical animal models for gene therapy of Wiskott-Aldrich syndrome, chronic granulomatous disease and Goucher disease. SB transposition occurs into chromosomes in a random manner, which is clearly undesired for human applications due to potential genotoxic effects associated with transposon integration. 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 such as a designer zinc finger. Targeted transposition could be a powerful method for safe transgene integration in human applications.

Selected Publications

Ivics, Z, Li, M.A., Mátés, L, Boeke, JD, Nagy, A, Bradley, A, Izsvák, Z. (2009) Transposon-mediated genome manipulations in vertebrates. Nature Methods 6(6), 415-422.

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Young-Ae Lee

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Genetics of Allergic Disease

The allergic diseases, particularly atopic dermatitis, food allergy, asthma, and hay fever, are among the most common chronic diseases in man. The prevalence of atopic diseases has increased to epidemic dimensions over the past decades. In the industrialized countries, 25-30% of the population are affected. Genetic and environmental factors interact to determine disease susceptibility, and family and twin studies indicate that the genetic contribution is substantial. Our group is using genetic and genomic approaches to identify genes and genetic variants that predispose to atopic dermatitis and atopy. The identification of the molecular pathways underlying allergic disease will provide novel targets for preclinical diagnosis, disease prevention, and therapeutic intervention.

Genomewide Association study reveals a common variant on chromosome 11q13 that is associated with atopic dermatitis

Atopic dermatitis is a chronic inflammatory skin disorder and a major manifestation of allergic disease. The molecular mechanisms underlying eczema are not fully understood. Skin barrier defect as well as systemic and cutaneous immune dysfunction in response to allergens or bacterial products are thought to play an important role.

To identify genetic variants contributing to atopic dermatitis, we conducted a genome-wide association study in 939 individuals with atopic dermatitis and 975 controls as well as 270 complete nuclear families with 2 affected siblings on Affymetrix Human mapping 500K and 5.0 arrays. SNPs consistently associated with atopic dermatitis in both discovery sets were then investigated in two additional independent replication sets totalling 2637 cases and 3957 controls (Figure 1). Highly significant association was found with a common sequence variant on chromosome 11q13.5 ($P_{combined}$ = 7.6 x 10^{-10}) in all 4 study groups. Approximately 13% of individuals of European origin are homozygous for the risk allele, and their risk of developing atopic dermatitis is 1.47 times that of noncarriers. Linkage disequilibrium analysis in Hapmap showed that rs7927894 is located in a 200 kb LD block containing a single gene called *C11orf30* (Figure 2). *C11orf30* encodes the nuclear protein EMSY which has been implicated in breast cancer susceptibility, chromatin modification, DNA repair, and transcriptional regulation. The potential involvement of *C11orf30* in multiple inflammatory and malignant epithelial diseases (atopic dermatitis, Crohn´s disease, and adenocarcinoma) strongly suggests a role for *C11orf30* in epithelial immunity, growth, and/or differentiation.

Finally, we provide a list of additional candidate genes. Further replication in independent cohorts, fine mapping and functional studies will be required to gain a better understanding of the physiological mechanisms underlying this common allergic disorder.

Towards the genetic prediction of childhood asthma

Asthma is a chronic inflammatory lung disease featuring intermittent airway obstruction triggered by environmental allergens, exercise or viral infections. The increasing prevalence of asthma and the lack of curative therapy underscores the need for effective disease prediction and prevention. We have performed the first genetic prediction study for asthma using the loss-of-function mutations in the filaggrin gene (*FLG*), which

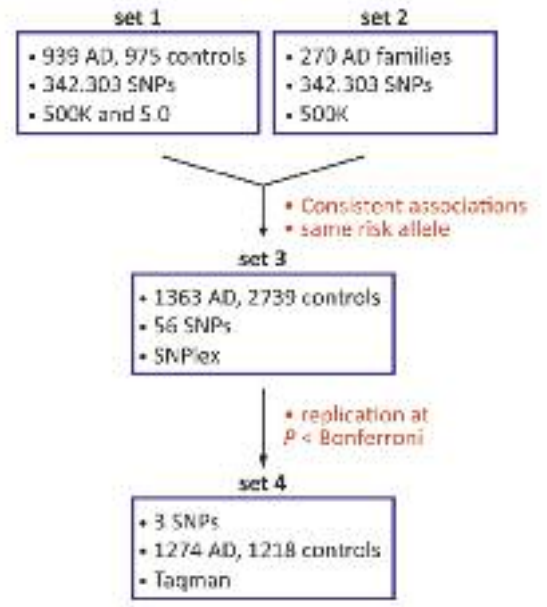

Figure 1. *Study design*

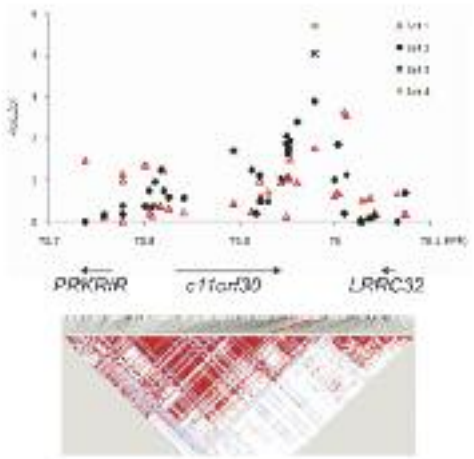

Figure 2. *Association results and LD structure in the AD associated region on 11q13.5. (a) Association results for all GWA markers tested in the region. Physical positions are based in the NCBI build 36. (b) Genes in the region from the UniGene database. (c) LD in the CEU Hapmap population. Disequilibrium coefficient values for Hapmap Phase II data (v. 22) were generated with Haploview. The lead SNP is indicated by a green line.*

were identified to be a strong genetic risk factors for eczema and eczema-associated asthma. We investigated in the population-based Multicenter Allergy Study (MAS cohort) whether the predictive value of the *FLG* mutations for asthma was related to infantile eczema. Furthermore, we investigated the role of allergic sensitization to food allergens which represents the earliest serologic marker for atopy and is a recognized risk factor for chronic asthma.

We found that, in infants with eczema and sensitization to food allergens within the first three years of life, the presence of a *FLG* loss of function mutation predicts the future development of asthma with a specificity and a positive predictive value of 100%. The combination of *FLG* mutations and early sensitization to food allergens, predicted a sizeable proportion (17.2%) of the infants with eczema who made the transition to asthma later in childhood. Furthermore, longitudinal pulmonary function measurements demonstrated that this subgroup of asthma children identified by the *FLG* mutations carried a poor prognosis with a steady decline in pulmonary function until puberty. Hence, this subgroup might particularly benefit from early prediction of the disease. Our findings indicate that assessment of the *FLG* carrier status could improve the prediction of eczema-associated asthma considerably. The magnitude of the predictive power surpasses the utility of mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* that account for only 2-4% of all breast cancer cases and for 15-20% among the high risk group of women with a strong family history and early onset of the disease in whom targeted genetic testing was recommended. Analogously, in the high risk group of children with eczema and early food sensitization the genotyping of the *FLG* mutations would identify 35.7% of future asthmatics.

In this study, we demonstrate that the determination of the *FLG* carrier status in infants with eczema and sensitization to food allergens within the first three years of life would allow the early prediction of asthma before the onset of symptoms and at a critical time of immune development when preventive interventions are likely to be effective. We suggest our findings could be of diagnostic and subsequent therapeutic utility.

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Cell Signaling and Mass Spectrometry

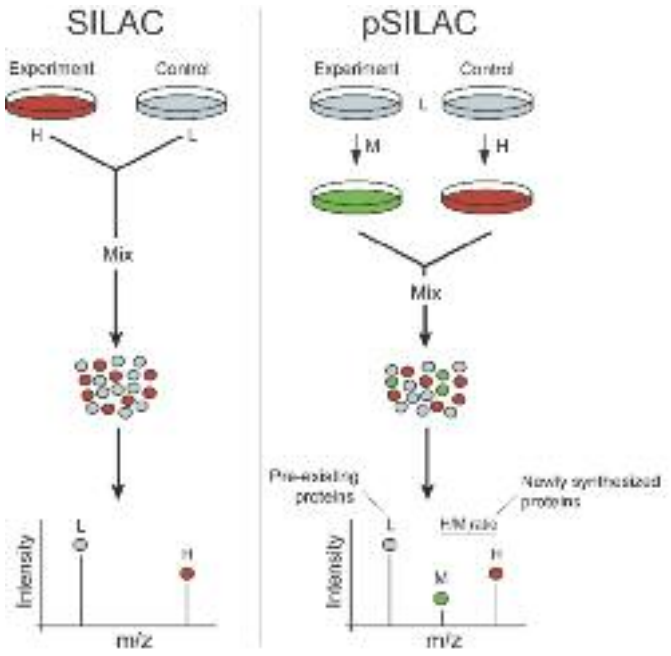
Proteins are the chief actors in almost every biological process. While we know a lot about the function of individual proteins there is little information about the system as a whole. Recent developments in mass spectrometry have dramatically improved the analytical power of this technology. We are using mass spectrometry-based quantitative proteomics to investigate cellular signaling at the protein level on a global scale. Main areas of research are posttranscriptional regulation of gene expression by microRNAs, protein-protein interaction in the context of neurodegenerative diseases and *in vivo* quantitative proteomics.

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 labeled by cultivating them in the presence of either normal or a heavy isotope–substituted amino acid, such as ^{13}C-labeled lysine (Figure, left panel). 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. Quantitative proteomics with SILAC has emerged as a very powerful approach to investigate signaling processes. We are using this technology as our central tool to address challenging questions in cell signaling.

pSILAC and microRNAs

Regulation of gene expression occurs at all stages from mRNA transcription to protein synthesis. It is now clear that translation itself is a regulated process with a central role in cellular physiology and a growing catalogue of human diseases. A prominent example are microRNAs: These small non-coding RNAs repress target genes by inhibiting translation or by inducing degradation of mRNAs. In mammals, microRNAs are predicted to control the activity of ~30% of all protein-coding genes. They have been shown to be involved in regulation of almost every cellular process investigated so far. In order to understand microRNA function it is crucial to investigate how they regulate protein production.

We developed pulsed SILAC (pSILAC) as a novel method to directly compare protein translation rates between two samples (Figure, right panel). Cells are first cultivated in standard growth medium with the normal light (L) amino acids. Concomitantly with differential treatment, cells are transferred to culture medium containing heavy (H) or medium-heavy (M) amino acids. All newly synthesized proteins will be made in the H or M form, respectively. Subsequently, both samples are combined and analyzed together. The abundance ratio of H versus M peptides reflects differences in translation of the corresponding proteins integrated over the pulse labelling incubation time. We employed pSILAC to measure changes in synthesis of several thousand proteins after misexpression of microRNAs (collaboration with the group of Nikolaus Rajewsky). Bioinformatic analysis of the data showed that a single microRNA can directly repress hundreds of targets and that this repression is typically rather mild. By comparing the pSILAC and the microarray data we were able to show that microRNAs directly repress translation of hundreds



The principle standard SILAC (left) and pulsed SILAC (right). *In standard SILAC, cells are completely labeled by cultivating them in growth medium containing light (L) or heavy (H) stable (i.e. non-radioactive) amino acids. After several cell generations, all proteins have uniformly incorporated the isotope label. Differentially labeld cells can be combined and analyzed together by mass spectrometry. The ratio of peak intensities reflects differences in protein abundance between both samples. In pSILAC, cells are growing in normal (i.e. light) medium. Upon differential treatment, cells are transferred to medium-heavy (M) of heavy (H) medium for pulse labelling. The ratio of heavy and medium-heavy peaks is a direct measure of the differences in protein synthesis between both experimental conditions.*

of mRNAs. We are currently extending this analysis to microRNAs involved in carcinogenesis. (Olivia Ebner, Björn Schwanhäusser)

Protein-protein interaction in neurodegenerative diseases

Identifying interaction partners is the key to protein function and can provide insight into disease mechanisms. Neurodegenerative diseases like Alzheimer's are frequently caused by accumulation of toxic protein species in neuronal cells. We are using quantitative mass spectrometry and network analysis to analyze protein-protein interactions (PPIs) involved in disease pathogenesis. A useful method to study PPIs is affinity purification using a bait molecule. However, such pull-down assays suffer from the trade-off between sensitivity and specificity: While more stringent purification conditions can remove some contaminating proteins they might also eliminate specific interaction partners with weak affinities and/or of low abundance. Quantitative proteomics solves this problem by comparing the abundance of proteins identified in a pull-down experiment with a suitable internal control. This allows the development of assays that can directly lead to understanding of biological systems. We are employing this strategy to identify interaction partners of proteins involved in neurodegeneration. Particularly, we are interested in identifying interactions affected by disease-associated mutations. (Fabian Hosp, Florian Paul)

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, fruit flies and zebrafish are attractive alternatives that are extensively used in many areas of biomedical research, especially in genetics and developmental biology. While SILAC has enormously improved quantitative proteomics in cultured cells, the method was not yet used in small animal models. We are currently extending this technology to three of the most important model organisms in biomedical research: *Caenorhabditis elegans*, *Drosophila melanogaster* and *Danio rerio*. (Matthias Sury, Marieluise Kirchner)

Selected publications

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microRNAs and 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 β-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.

The mechanisms governing gene expression patterns integrate both transcriptional activation, post-transcriptional gene silencing, and post-translational modifications. In the last decade the complex picture of gene regulation has been extended by the discovery of microRNAs. MicroRNAs are short, approximately 22 nucleotide long non-coding RNAs which are thought to be involved in a number of evolutionary conserved regulatory pathways. Many published reports have clearly illustrated a role for individual microRNA sequences in developmental timing, apoptosis, proliferation, differentiation, and organ development. However, in light of the many advances in the fields of microRNAs and RNA interference, many questions remain concerning the functional role of microRNAs in tissues like the pancreatic islet. Our work aims to test the hypothesis that microRNAs expressed in the islet play an important role in the development and function of pancreatic β-cells through their ability to regulate gene expression. Many direct targets of microRNAs expressed in the pancreatic islet have never been studied with respect to β-cell biology and it is important to understand how they contribute to islet function. Using newly developed mouse models, a molecular understanding of the exact nature of microRNAs is necessary to develop therapeutic strategies for the treatment of metabolic diseases like diabetes.

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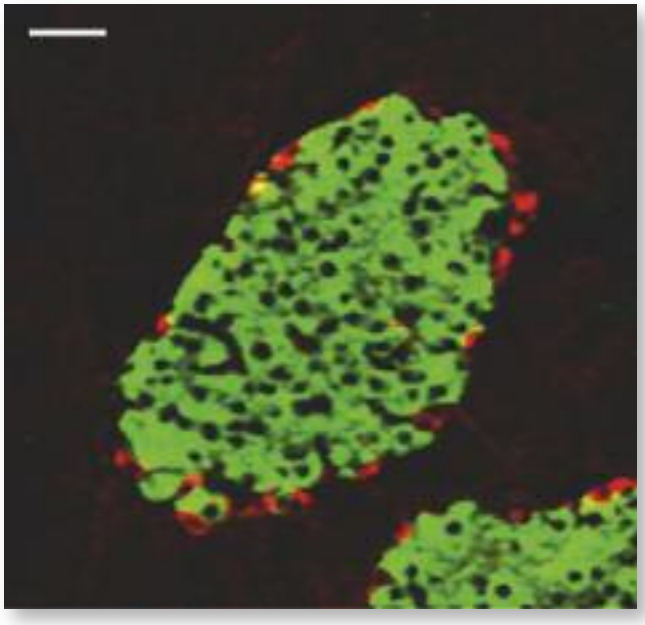
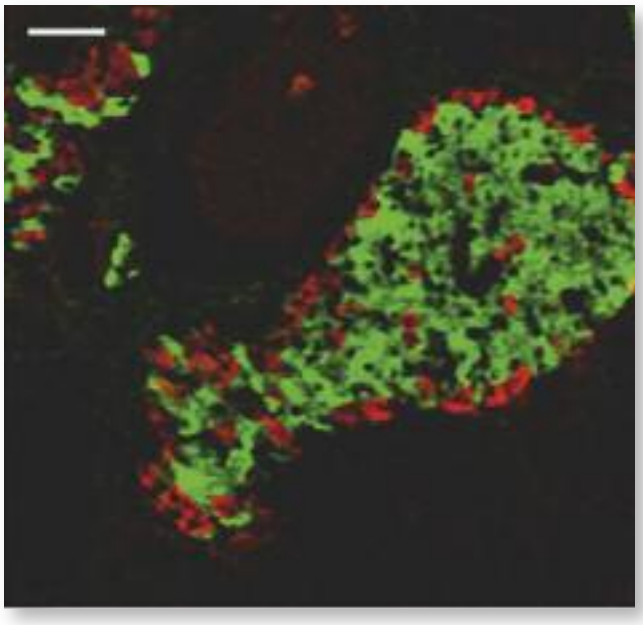
* Start of the group: August 2008





Decreased β-cell mass in miR-375KO pancreatic islets. Representative sections of pancreas from 10-week-old a)wild type and b)miR-375KO mice visualized by immunofluorescence after staining with insulin (green) and glucagon (red). Bar represents 25μm.

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Mathematical Modelling of Cellular Systems

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 signaling 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 signaling and gene-regulatory networks since these are critically involved in the prediction of the efficiency and possible side-effects of drugs.

Modelling of IKK/ NF-κB signaling

Bente Kofahl

In this collaborative project with the group of Claus Scheidereit at the MDC we aim for a systems level understanding of the IKK/NF-κB signaling 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 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 signaling branches. In a first step we have developed a mathematical model of the non-canonical IKK/NF-κB pathway, which is currently refined in an iterative cycle of experiments and modelling and extended towards canonical signaling.

Wnt/ β-catenin signaling

Uwe Benary, Bente Kofahl

Wnt/β-catenin signaling plays an important role in development and tissue homeostasis. Its deregulation is associated with various diseases and cancer. We use a mathematical model of the Wnt/β-catenin pathway to study the effect of β-catenin mutations critically involved in hepatocellular carcinoma. In collaboration with Rolf Gebhardts group (Leipzig) we are interested in the impact of the mutations on the dynamics of the signaling pathway and the expression of target genes in hepatocytes.

Secretariat
Sonja Giering
Petra Haink

*part of the period reported

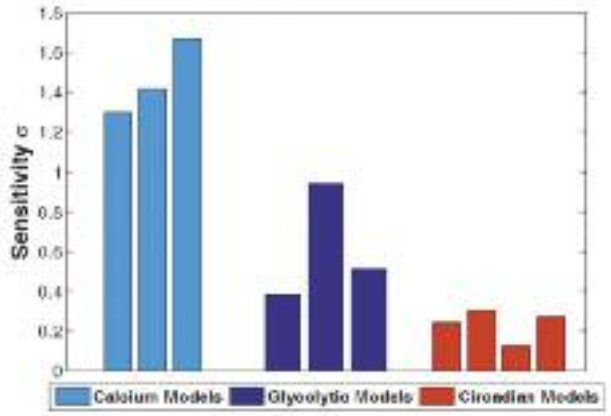
In other projects we investigate cell-type specific differences in Wnt/β-catenin signaling, analyse the effect of transcriptional feedbacks (e.g. via Axin, β-TrCP/ HOS) and that of cross-talks of Wnt/β-catenin signaling to other pathways, most importantly NF-κB.

Robustness of cellular rhythms

Katharina Baum, Antonio Politi

Rhythmic phenomena are widespread in biology and many examples 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 analyse specific examples of oscillations. By comparing various well-established mathematical models for calcium, glycolytic and circadian rhythms we could already show that the sensitivity of the oscillatory period against perturbations strongly depends on the underlying oscillatory mechanism. We now ask the question to what extent robustness is determined by local kinetic parameters or the topology of the systems. In our comparison, we find models for calcium oscillations to be very sensitive, those for circadian rhythms very robust. These findings correspond well to the biological functions of the rhythms since for calcium oscillations a period-encoded signal-transduction was shown, whereas circadian oscillations generate an internal time information. In addition, the effect of the feedback type on the sensitivity is studied in core models.



Comparison of the period sensitivity of cellular oscillators. Shown is the overall period sensitivity of ten mathematical models describing three cellular rhythms. Models for calcium oscillations are very sensitive, those for circadian rhythm are very robust. Models for glycolytic oscillations show an intermediate sensitivity.

Selected publications

J. Wolf, S. Dronov, F. Tobin & I. Goryanin (2007), The impact of the regulatory design on the response of EGFR-mediated signal transduction towards oncogenic mutations. FEBS Journal 274, 5505-5517.

J. Wolf, S. Becker-Weimann & R. Heinrich (2005), Analysing the robustness of cellular rhythms, IEE Syst. Biol. 2(1), 35-41.

S. Becker-Weimann, J. Wolf, H. Herzel & A. Kramer (2004), Modeling feedback loops of the mammalian circadian oscillator. Biophysical Journal 87, 3023-3034.

Markus Landthaler

Structure of the Group

Group Leader
Dr. Markus Landthaler

Technical Assistants
Julia Kretschmer

Scientists
Alexander Baltz
Lea Gregersen
Kerstin Baethge

RNA Biology and Post-transcriptional regulation

Our main interest is the understanding of post-transcriptional regulatory networks controlling gene expression in humans. In cells 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 post-transcriptional 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-immunoprecipitation approach (PAR-CLIP) in combination with massively parallel sequencing to identify functional RNA-protein interactions at single-nucleotide resolution.

Post-transcriptional regulation by RNA-binding proteins

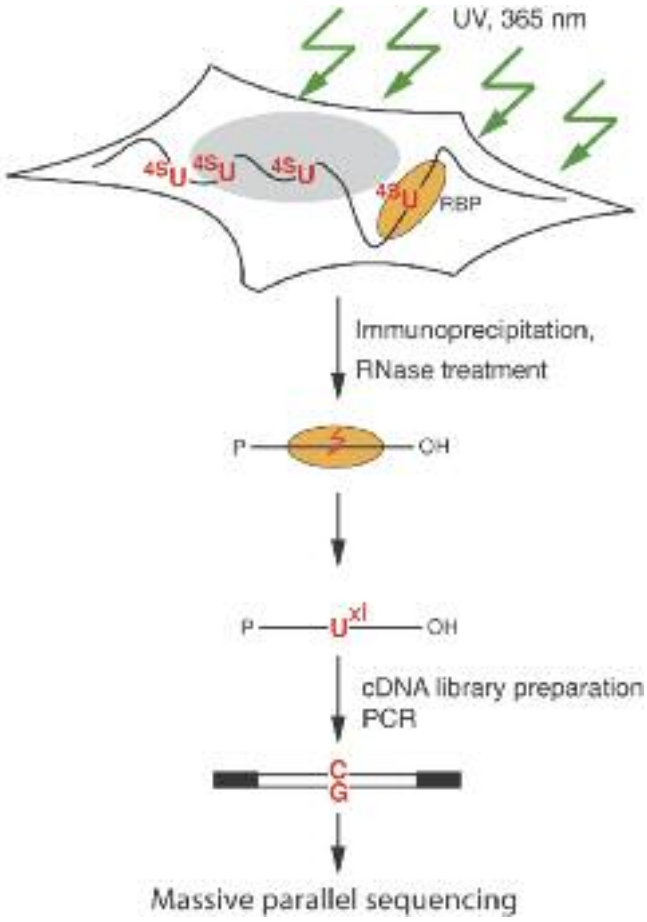
Mammalian genomes encode several hundred RNA-binding proteins, each containing one or multiple domains able to recognize target mRNA in a sequence- and/or structure-dependent manner. The association of these proteins with RNA regulates the biogenesis and translation of RNA. For a large number of RNA-binding 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

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 crosslinking-immunoprecipitation approach to define functional interactions of helicases and RNA that are typically transient in nature.

The identification of RNA target sites provides a foundation for biochemical and reverse genetic approaches to investigate the remodeling mechanism of ribonucleoprotein complexes by helicases. These studies will provide insights into the determinants of target RNA selection, functional interactions with other RNA-interacting proteins and the physiological role of RNA helicases.



PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation)

Photoactivatable 4-Thiouridine (4SU), when provided to cultured cells, is incorporated into nascent RNAs and crosslinked to RNA-interacting proteins by long-wave UV irradiation. The protein of interest is immunoprecipitated and the covalently bound RNA reduced to a short protected segment by ribonuclease treatment. In contrast to other methods, the sites of crosslinking are identified by mapping T to C transitions residing in the cDNA of libraries prepared from RNA crosslinked to a specific RNA-interacting protein.

Regulation of gene expression by non-coding RNAs

Non-coding RNA (ncRNA) are functional RNAs that are not translated into proteins. Recent evidence indicates that sizable regions of mammalian genomes are transcribed into ncRNAs, which are alternatively spliced and/or processed into smaller RNAs. microRNAs, the most prominent class of small ncRNAs, mediate destabilization and inhibition of translation target mRNAs.

We are using next generation sequence technology to discover large ncRNAs in human cells lines. To obtain insights into the function of identified ncRNAs we deplete these RNAs by RNA interference and characterize associated proteins by photo-crosslinking and mass spectrometry.

Selected Publications

Hausser J*, Landthaler M*, Jaskiewicz L, Gaidatzis D, Zavolan M. (2009) Relative contribution of sequence and structure features to the mRNA-binding of Argonaute/miRNA complexes and the degradation of miRNA targets. Genome Res 19. 2009-2020.

Zhang Y, Landthaler M, Schlussman SD, Yuferov V, Ho A, Tuschl T, Kreek MJ.(2009) Mu opioid receptor knockdown in the substantia nigra/ventral tegmental area by synthetic small interfering RNA blocks the rewarding and locomotor effects of heroin. Neuroscience 158, 474-483

Yi R, Pasolli HA, Landthaler M, Hafner M, Ojo T, Sheridan R, Sander C, O'Carroll D, Stoffel M, Tuschl T, Fuchs E. (2009) DGCR8-dependent microRNA biogenesis is essential for skin development. PNAS 106, 498-502.

Landthaler M, Gaidatzis D, Rothballer A, Chen PY, Soll SJ, Dinic L, Ojo T, Hafner M, Zavolan M, Tuschl T. (2008) Molecular characterization of human Argonaute-containing ribonucleoprotein complexes and their bound target mRNAs. RNA 14, 2580-2596.

Landthaler M, Yalcin A, Tuschl T. (2004) The human DiGeorge syndrome critical region gene 8 and Its D. melanogaster homolog are required for miRNA biogenesis. Curr. Biol.14, 2162-7.

Cancer Research Program

Krebsforschung

Coordinator: Claus Scheidereit

Signaling Pathways, Cell and Tumor Biology

Coordinator: Achim Leutz

Structural and Functional Genomics

Coordinator: Udo Heinemann

Tumor Immunology

Coordinator: Martin Lipp

Cancer Research Program

Krebsforschungsprogramm *Claus Scheidereit, Achim Leutz, Martin Lipp, Udo Heinemann*

As a collective term, cancer denotes a variety of malignant neoplastic diseases that originate from different organs through the accumulation of multiple mutations in the genome. These alterations affect the functioning of sets of genes which control the normal behavior of cells in their tissue context. It is a shared feature of the different types of cancer cells that they escape the natural surveillance of growth control by their environment: cancer cells proliferate in an uncontrolled manner, they evade their elimination by natural cell death, escape the control by the immune system and finally disperse in the body by migrating through the blood and lymph system (metastasis) to form secondary tumors. The objective of the Cancer Research Program is to understand at the molecular and cellular level how cancer develops and progresses in order to provide the basis for improved diagnosis and, ultimately, treatment of cancer.

The human genome contains around 23,000 protein encoding genes, a number of which are of particular importance for the regulation of cellular behavior. In cancer cells, many of these crucial genes are repeatedly found to have acquired genetic mutations. These affected genes are categorized as either "oncogenes" or "tumor suppressor genes", depending on their mode of action. They code for regulatory and structural proteins that control critical processes, such as cell proliferation, differentiation, apoptosis, cell migration or angiogenesis. Often, cancer cells have accumulated sets of mutated genes for these different functions. Many of these genes are also active during embryogenesis or during the differentiation of distinct cell types. This is why tumor cells appear to share characteristics of embryonic cells.

The research groups of the MDC Cancer Research Program work in the fields signal transduction and growth control, structural genomics and tumor immunology. The broad expertise in basic biomedical as well as clinical disciplines allows to investigate the causes and the emergence of cancer and to find rational treatments. Cancer studies are conducted in close collaboration with clinical research groups of the Charité/ Medical Faculty Berlin. The aim of the Program is to discover and to characterize genes that

Als Sammelbegriff bezeichnet Krebs eine Bandbreite unterschiedlicher, bösartiger Tumor-Erkrankungen, die in verschiedenen Organen des Körpers durch Anhäufungen von Mutationen im Genom entstehen können. Diese Mutationen verändern die Funktion von Genen, die das normale Verhalten der Zellen in ihrem Gewebsverband steuern. Eine gemeinsame Eigenschaft der Zellen verschiedener Krebstypen ist, dass sie sich der natürlichen Wachstumskontrolle durch ihre Umgebung entziehen. Krebszellen teilen sich spontan und unreguliert, entgehen dem natürlichen Zelltodprogramm und der Abwehr durch das Immunsystem und können den Zellverband über Blut- und Lymphkreislauf verlassen, um sich an anderer Stelle als Metastasen anzusiedeln. Im Forschungsprogramm des MDC soll auf molekularer und zellulärer Ebene verstanden werden, wie Krebs entsteht, um die Grundlage für eine verbesserte Diagnose und Therapie von Krebserkrankungen zu schaffen.

Das menschliche Genom enthält etwa 23000 Proteinkodierende Gene, von denen eine Anzahl für die Steuerung des Verhaltens der Zelle im Gewebeverband verantwortlich ist. Viele dieser Gene finden sich regelmäßig in mutierter, also genetisch veränderter Form, in Krebszellen wieder. Diese Klasse von Genen wird begrifflich, entsprechend ihrer Wirkungsweise, in „Onkogene" (potentiell krebsfördernd) und „Tumor-Suppressor-Gene" (potentiell krebshemmend) eingeteilt. Alle kodieren für Proteine, die eine Funktion in der Steuerung von Prozessen wie Zellproliferation, Zelldifferenzierung, Apoptose, Zellmigration oder Angiogenese haben. Krebszellen weisen häufig Mutationen mehrerer solcher Gene auf. Viele dieser Proteine sind normalerweise während der Embryogenese und ebenso später bei der Regeneration bestimmter Zelltypen aktiv. Deshalb haben Tumorzellen auch zahlreiche Eigenschaften mit embryonalen Zellen gemeinsam.

Die Forschergruppen des Krebs-Forschungsprogramms des MDC arbeiten auf den Gebieten der Signalübertragung, Wachstumskontrolle, Strukturbiologie des Genoms und der Tumor-Immunologie. Experimentell-theoretische und klinische Expertisen werden systematisch eingesetzt, um die Ursachen und den Entwicklungsverlauf von Krebs zu verstehen und zielgerichtete Therapien zu entwickeln. Die Studien werden in enger Zusammenarbeit mit klinisch orientierten Forschergruppen der Medizinischen Fakultät Berlin (Charité) durchgeführt. Das Ziel des Krebs-Forschungsprogramms des MDC besteht darin, Gene zu identifizieren und zu untersuchen, die für die Entstehung von Krebs verantwortlich sind und zu ermitteln, welche Rolle deren Gen-

are responsible for the emergence of cancer and to determine the function of these gene products in cellular processes and disease progression. This understanding will be crucial for the development of new cancer treatments.

Signaling pathways, Cell Biology, and Cancer

The research in the program "Signaling pathways, Cell Biology and Cancer" aims to unravel the molecular basis underlying tumor formation. A focus is made on the study of de-regulated and normal function of oncogenic genes and on the cellular pathways and signals that regulate their activity. Thus, the research field covers the normal function of crucial regulatory systems during development or cell differentiation as well as the role in epithelial cancer and metastasis, in leukemia or lymphoma cells.

The program is further strengthened by investigations of important fundamental cell biological processes, including embryonic stem cell biology, cell cycle regulation, protein degradation pathways and regulation of chromatin by epigenetic mechanisms.

Cancer and metastasis

The process of metastasis is the major risk factor in cancer progression. Tumors can be classified into different categories according to how they infiltrate surrounding tissues. However, these parameters do not currently provide a method to predict the likelihood of tumor relapse. Currently, one of the best signs of an unfavorable disease course is lymph node infiltration. Patients with this diagnosis may be treated by adjuvant chemotherapy or radiation, yet more than 70 % of them suffer from a loss of quality of life rather than experiencing any benefits from adjuvant treatment. This situation could be greatly improved by a comprehensive understanding of the molecular mechanisms of tumor progression and metastasis. That would go hand-in-hand with the identification of reliable prospective markers that can be used to predict the likelihood of metastasis.

The group of **Peter M. Schlag**, in collaboration with **Walter Birchmeier**, has identified a previously uncharacterized gene that encodes the protein MACC1

produkte bei zellulären Vorgängen und im Krankheitsverlauf spielen. Ein solches Wissen ist eine entscheidende Voraussetzung für die Entwicklung zukünftiger Behandlungsverfahren.

Signalübertragungsketten, Zellbiologie und Krebserkrankungen

Die Forschung in diesem Bereich versucht, die molekularen Grundlagen der Tumorentstehung zu entschlüsseln. Ein Schwerpunkt ist die Untersuchung der deregulierten und normalen Wirkungsweise von Krebs-assoziierten Genen und der zellulären Signalwege, die deren Aktivität steuern. Es wird dabei die normale Funktion der entscheidenden Regulationssysteme während der Embryonalentwicklung oder Zelldifferenzierung als auch deren Rolle in Karzinomen und bei Metastasenbildung, in Leukämien oder Lymphomen untersucht. Das Programm wird durch die Erforschung wichtiger grundlegender zellbiologischer Themen, wie embryonale Stammzellbiologie, Steuerung des Zellzyklus, Kontrolle der Proteinstabilität und epigenetische Regulation der Chromatinstruktur verstärkt.

Krebs und Metastasierung

Die Bildung von Metastasen ist der dominierende Risikofaktor im Fortschreiten einer Krebserkrankung. Man kann Tumore danach klassifizieren, in welcher Weise sie umgebendes gesundes Gewebe infiltrieren. Leider kann man daraus noch keine zuverlässigen Vorhersagen für die Wahrscheinlichkeit eines Rückfalls nach einer Krebstherapie ableiten. Die beste Vorhersage eines ungünstigen Krankheitsverlaufes resultiert gegenwärtig aus dem Nachweis von Infiltrationen der Lymphknoten durch Krebszellen. Bei diesen Patienten kann Chemotherapie oder Bestrahlungsbehandlung eingesetzt werden, jedoch führt dies bei 70% der Behandelten eher zu einer erheblichen Verschlechterung der Lebensqualität als zu wirklichen Behandlungserfolgen. Diese Situation könnte erheblich durch eine umfassende Kenntnis der molekularen Mechanismen der Tumorprogression und Metastasierung verbessert werden. Dies würde es ermöglichen, prognostisch zuverlässige Tumormarker zu identifizieren, mit denen die Tendenz zur Metastasierung nachgewiesen werden kann.

*Die Gruppe von **Peter M. Schlag** hat in Zusammenarbeit mit **Walter Birchmeier** ein zuvor noch nicht charakterisiertes Gen identifiziert, welches das Protein MACC1 (Metastasis-Associated in Colon Cancer, publiziert in Nature Medi-*

(metastasis-associated in colon cancer-1; published in Nature Medicine) as a key regulator for HGF and Met mediated metastasis of colon cancer. MACC1 promotes proliferation, invasion, and HGF-induced scattering of colon cancer cells in culture and tumor growth and experimental metastasis in mouse models. Currently, MACC1 is clinically evaluated as a promising predictive factor for colon cancer. The group of **Walter Birchmeier**, in collaboration with **Peter M. Schlag,** has also identified genes whose expression correlates with the occurrence of metastasis in colon cancer. Using microarray transcript profiling, a signature of 115 genes was found whose expression can distinguish non-metastatic and metastatic primary colorectal carcinomas. The expression of one of the signature genes BAMBI, promotes experimental metastasis in mice and correlates with the survival of patients. BAMBI is a TGFβ inhibitor whose expression is controlled by the β-catenin/Bcl9-2 signaling pathway.

A high proportion of bone tumors metastasize. The group of **Achim Leutz** identified genes that are dysregulated in human osteosarcomas and are involved in osteosarcomagenesis in experimental animals.

E2F3 is essential for cellular division. Strong expression of this transcription factor leads to the development of tumors in humans. Through interaction studies new co-activators were identified in the lab of **Ulrike Ziebold**. In the future these co-activators may be used for targeted tumor therapies. In addition, genetically modified mouse strains harbouring pRB/E2F3 mutations are used since these mice develop metastatic medullary thyroid cancers. With the aid of these tumors 123 transcripts were identified, many of which are also upregulated in human metastasis. The clinical relevance of these markers is currently under investigation.

The molecular and functional analysis of cell adhesion and signaling is of fundamental importance in development and tumor progression and is a major focus of the laboratory of **Walter Birchmeier**. Previously, the lab has defined functions of E-cadherin/β-catenin at the cell periphery which mediate adhesion between epithelial cells and prevent invasion and metastasis. However, β-catenin is also a cen-

cine) kodiert. MACC1 ist ein Hauptregulator der durch HGF und Met-vermittelten Metastasierung bei Dickdarmkrebs. Es fördert Wachstum, Invasion und HGF-vermittelte Streuung von kultivierten Darmkrebszellen, als auch Tumorwachstum und experimentelle Metastasierung in Mausmodellen. Zur Zeit wird MACC1 als vielversprechender Tumormarker für Dickdarmkrebs klinisch evaluiert.

*In Kooperation mit **Peter M. Schlag** hat die Gruppe von **Walter Birchmeier** ebenfalls eine Reihe von Genen gefunden, deren Expressionshöhe mit dem Auftreten von Metastasen bei Dickdarmkrebs korreliert. Durch Ermittlung von Genexpressionsprofilen anhand von Mikroarrays wurde eine Gruppe von 115 Genen identifiziert, mit deren Expressionsverhalten nicht-metastasierende von metastasierenden kolorektalen Karzinomen unterschieden werden können. Die Expression eines dieser Gene, BAMBI, erhöht die experimentelle Metastase im Mausmodell und korreliert mit einer schlechten Prognose bei Darmkrebspatienten. BAMBI hemmt TGF und seine Expression wird durch den β-Catenin/Bcl9-2 Signalweg gesteuert.*

*Knochentumoren weisen eine hohe Metastasierungstendenz auf. Die Gruppe um **Achim Leutz** hat Gene identifiziert, die in menschlichen Osteosarkomen dereguliert sind und an der Entstehung von Osteosarkomen in Versuchstieren beteiligt sind.*

*E2F3 ist für die Teilung von Zellen wichtig. Eine hohe Expression dieses Transkriptionsfaktors in Geweben korreliert mit der Entstehung von Tumoren beim Menschen. Durch Interaktionsanalysen konnten im Labor von **Ulrike Ziebold** neue Koaktivatoren von E2F3 identifiziert werden. Diese Koaktivatoren könnten künftig für gezielte Tumortherapien benutzt werden. Zudem werden genetisch manipulierte Mäuse mit pRB/E2F3-Mutationen verwendet. In diesen Mäusen entstehen Metastasen des medullären Schilddrüsenkarzinoms. Mit Hilfe dieser Tumoren konnten 123 Transkripte nachgewiesen werden, von denen viele auch in menschlichen Metastasen hochreguliert werden. Die klinische Bedeutung dieser Marker wird gegenwärtig weiter untersucht.*

*Die molekulare und funktionelle Analyse der Zelladhäsion und der daran beteiligten Signalübertragungsketten ist von grundlegender Bedeutung für die Entstehung und Weiterentwicklung von Tumoren; sie ist ein wesentlicher Schwerpunkt der Forschungsgruppe von **Walter Birchmeier.** Durch das Labor wurden bestimmte Eigenschaften des E-Cadherin/β-Catenin-Systems an der Zellperipherie identifiziert, durch die eine Adhäsion der Epithelzellen bewirkt*

tral component of the canonical Wnt signaling pathway. β-Catenin may translocate from the periphery to the nucleus, bind to the transcription factors LEF/TCF and activate gene expression. In the present research period, cell type and tissue specific conditional gene ablation of β-catenin revealed intricate connections between the Wnt and BMP signaling pathways in the development of limbs, the central nervous system, the heart, and in tumor development. In addition to Wnt signaling, there is a long-standing interest in the biochemistry of scatter factor/hepatocyte growth factor (SF/HGF) and Met receptor signaling. Met signaling was found to regulate wound healing, and its downstream signal transducer Gab1 is required to mediate cell-context specific effects of Met.

Hematopoiesis, leukemia, and lymphoma

Stem cells in the bone marrow continuously replenish huge numbers of exhausted blood cells, including erythrocytes, granulocytes, macrophages, and lymphoid cells. In experimental hematology, cells of different blood cell lineages and maturation stages can be distinguished by cell surface markers and can be isolated by cell sorting for prospective studies. Differentiation of hematopoietic cells from progenitors may then be recapitulated in tissue culture. Moreover, murine hematopoietic stem cell transplantation can be combined with experimental genetics, two powerful tools to explore somatic stem cell biology and cell differentiation control.

Deregulation of hematopoietic stem cell biology and cell differentiation pathways may cause diseases. Transcription factors are at the core of pathways that determine self-renewal versus differentiation and maintain cell function. The common view is that key transcription factors navigate hematopoiesis along hierarchical cell differentiation routes and that mutations that disrupt their normal functions are central to disease development. Unraveling the genetics and epigenetics of somatic stem cell biology and differentiation are prerequisites for the design of novel therapies. MDC research groups have accomplished major achievements towards understanding basic mechanisms in hematopoietic stem cell and precursor cell biology in the last years.

und so das Eindringen von Zellen und Metastasierung verhindert wird. β-Catenin ist jedoch auch ein zentraler Faktor in der kanonischen Wnt-Signalübertragungskette. Das Protein kann von der Zellperipherie in den Zellkern wandern, dort an die Transkriptionsfaktoren LEF/TCF binden und damit die Expression von Genen auslösen. Im Berichtszeitraum gelang es, durch zell- bzw. gewebespezifische Ausschaltung des β-Catenin Gens komplexe Wechselbeziehungen zwischen den Wnt- und BMP-Signalübertragungsketten bei der embryonalen Entwicklung der Extremitäten, des zentralen Nervensystems, des Herzens und bei der Tumorentstehung nachzuweisen. Neben der Wnt-Signalkette ist die Forschungsgruppe von ***Walter Birchmeier*** *seit langem an der Biochemie des Scatter Factor/Hepatocyte Growth Factor (SF/HGF)-Systems und der Met-Rezeptor Signalübertragung interessiert. Das letztere System wurde als Regulator der Wundheilung identifiziert und es wurde gefunden, dass ein in der Signalkette weiter abwärts gelegener Signalüberträger, Gab1, für die Vermittlung Kontext-spezifischer Effekte von Met notwendig ist.*

Hämatopoese, Leukämie und Lymphome

Stammzellen des Knochenmarks müssen permanent große Mengen verbrauchter Blutzellen, wie Erythrozyten, Granulozyten, Makrophagen und lymphatische Zellen, neu bilden. In der experimentellen Hämatologie können die verschiedenen Zelltypen und deren Reifungsgrade unterschieden und im Zellsortierer isoliert werden, wodurch prospektive Studien ermöglicht werden. Die Differenzierung der hämatopoetischen Zellen aus deren Vorläuferzellen kann dann in der Gewebskultur nachvollzogen werden. Weiterhin kann die Transplantation hämatopoetischer Stammzellen in der Maus mit experimenteller Genetik kombiniert werden, zwei wirkungsvolle Werkzeuge, um die Biologie der Stammzellen und die Kontrolle der Zelldifferenzierung zu erkunden.

Eine Störung der hämatopoetischen Stammzellbiologie und der Signalwege, die zelluläre Differenzierung steuern, kann Erkrankungen zur Folge haben. Transkriptionsfaktoren sind für die Signalwege, die über Selbsterneuerung oder Differenzierung entscheiden und zelluläre Funktionen aufrecht erhalten, von zentraler Bedeutung. Es wird allgemein angenommen, dass bestimmte Transkriptionsfaktoren die Hämatopoese entlang hierarchischer Zelldifferenzierungs-Routen navigieren und dass Mutationen, die deren normale Aktivität unterbrechen, zur Krankheitsentwicklung entscheidend beitragen. Eine Entschlüsselung der Genetik und Epigenetik der somati-

In the lab of **Achim Leutz**, the transcription factor C/EBPβ was found to be regulated at the level of translation initiation. Growth signals and nutritional conditions determine whether long or short C/EBPβ isoforms are generated from its messenger RNA. Genetic manipulation of mice now demonstrated the physiological importance of translational switching in C/EBPβ expression. Mice that express too much of the truncated C/EBPβ isoform develop osteoporosis due to hyperactive osteoclasts, a cell type that degrades bone and that is derived from myeloid blood cells. Mice that can not switch to the truncated isoform at all and that express only long C/EBPβ are defective for liver regeneration (published in EMBO Journal and in Genes & Development). Thus, translational regulation of C/EBPβ isoforms is important in homeostasis and regeneration and the pathways and mechanisms uncovered in these studies may provide novel pharmacological targets.

DNA methylation is a dynamic epigenetic mark that undergoes extensive changes during differentiation of self-renewing stem cells. Moreover, altered DNA methylation is a hallmark of cancer, and drugs targeting methylating enzymes are used in cancer therapy. **Frank Rosenbauer**'s research group revealed very recently a crucial role of DNA methylation in the control of both normal and cancerous hematopoietic stem cells (published in Nature Genetics). Their results identify DNA methylation as an epigenetic mechanism to block premature activation of predominant differentiation programs in stem cells as a key prerequisite to maintain multipotent developmental options and self-renewal. These data suggest that competing stem cell programs require different methylation dosage-dependent control mechanisms, and identify CpG methylation as a shared epigenetic program in the control of normal and neoplastic stem cells.

Numerous physiological and pathological processes are regulated by transcription factors of the NF-κB family and by IκB kinases (IKK), which control NF-κB activity. In normal physiology, transiently activated NF-κB transcription factors are central regulators of the adaptive and innate immune response and of inflammation. However, pathological conditions can

schen Stammzellbiologie und Differenzierung ist eine Voraussetzung für die Entwicklung neuer Therapien. Forschergruppen des MDC haben in den letzten Jahren bedeutende Beiträge zum Verständnis grundlegender Mechanismen in der Biologie hämatopoetischer Stamm- und Vorläuferzellen geleistet.

*Im Laboratorium von **Achim Leutz** wurde gefunden, dass der Transkriptionsfaktor C/EPBβ auf Ebene der Translationsinitiation reguliert wird. Wachstumssignale und Nährstoffbedingungen bestimmen, ob lange oder kurze C/EBPβ Isoformen von der Boten-RNA gebildet werden. Durch genetische Manipulationen in der Maus konnte jetzt die physiologische Bedeutung der translationalen Umschaltung in der Expression von C/EBPβ Isoformen nachgewiesen werden. Mäuse, die zu viel der verkürzten Isoform exprimieren, entwickeln Osteoporose. Der Grund sind hyperaktive Osteoklasten, ein Zelltyp, der Knochen abbaut und von myeloiden Blutzellen abstammt. Mäuse, die nicht zu der verkürzten Isoform umschalten können und ausschließlich langes C/EBPβ bilden, weisen einen Defekt in der Regeneration der Leber auf (publiziert in EMBO J. und Genes & Development). Die translationale Regulation der C/EBPβ Isoformen spielt also für Homeostase und Regenerierung eine wichtige Rolle und die in diesen Studien entdeckten Mechanismen könnten zu neuen pharmakologische Zielstrukturen führen.*

*DNA Methylierung ist eine dynamische epigenetische Markierung, die während der Differenzierung und Selbsterneuerung von Stammzellen ausgeprägten Veränderungen unterliegt. Weiterhin ist veränderte DNA Methylierung ein Kennzeichen von Krebszellen. Substanzen, die gegen methylierende Enzyme gerichtet sind, werden in Krebstherapien verwendet. Die Forschungsgruppe von **Frank Rosenbauer** hat kürzlich eine entscheidende Rolle der DNA-Methylierung bei der Kontrolle sowohl normaler als auch krebsartiger hämatopoetischer Stammzellen aufgedeckt (publiziert in Nature Genetics). Durch diese Ergebnisse wird DNA Methylierung als ein epigenetischer Mechanismus bestimmt, der eine verfrühte Aktivierung von Differenzierungsprogrammen in Stammzellen blockiert, um die Voraussetzung für multipotente Entwicklungsoptionen und Selbsterneuerung aufrecht zu erhalten.*

Zahlreiche physiologische und pathologische Vorgänge werden durch Transkriptionsfaktoren der NF-κB-Familie und durch IκB Kinasen (IKK), die NF-κB regulieren, kontrolliert. Normalerweise sind kurzzeitig aktivierte NF-κB-Transkriptionsfaktoren zentrale Regulatoren der angeborenen und der adaptiven Immunreaktion. Viele pathologische

lead to an aberrant constitutive activation, refractory to the normal homeostatic control. Previously, the group of **Claus Scheidereit** in collaboration with **Bernd Dörken** has shown that constitutive NF-κB plays a key role in tumor cell survival in Hodgkin's lymphoma, where it promotes cell cycle progression and blocks apoptosis (programmed cell death). The activation of NF-κB is also thought to be one of the potential causes for tumor cell resistance to chemo and radiation therapy. However, it was not understood how NF-kappaB is switched on by DNA damage, which is caused by these treatments in order to ablate tumor cells by apoptosis. The laboratory of **Claus Scheidereit** has now succeeded in identifying the start signal for an NF-κB dependent cell survival program (published in Molecular Cell). PARP-1, which detects DNA strand breaks within seconds, forms poly(ADP-ribose), which acts as a scaffold molecule and rapidly sequesters IKKγ, the kinase ATM as well as other signaling molecules in the nucleus. Subsequent chemical modifications imposed on IKK then trigger NF-κB activation and protection against apoptosis. These findings have implications for clinical studies using PARP-1 inhibitors in tumor therapy.

In embryonic development, NF-κB is required for the formation of epidermal structures, including hair follicles, eccrine glands or teeth. Using murine models it could now been shown how NF-κB and Wnt/β-catenin signaling pathways are integrated to control in a reciprocal manner the formation and growth of follicle placodes in early embryonic development (published in Development Cell). A cross-talk of NF-κB and Wnt/-β-catenin may also be of importance in epithelial tumors, where these factors are activated.

In addition to apoptotic cell death, cellular senescence has been recognized as another cellular safeguard program that responds to cellular insults such as oncogenic activation or DNA damage by locking the cell into a terminal arrest at the G1 phase of the cell-cycle. The group of **Clemens Schmitt** has made a landmark discovery by showing that the anti-cancer senescence program prevents the Ras oncoprotein from induction of lymphoma. The mechanism they discovered involves an enzyme that was already known to shut down genes by histone methylation of

Bedingungen können jedoch zu einer abweichenden permanenten Aktivierung von NF-κB führen, die der normalen homöostatischen Kontrolle entgeht. Die Gruppe von ***Claus Scheidereit,*** *in Zusammenarbeit mit* ***Bernd Dörken****, hatte bereits nachgewiesen, dass die konstitutive Aktivierung von NF-κB eine kritische Rolle bei dem Überleben von Tumorzellen des Hodgkin-Lymphoms spielt, indem es den Zellzyklus antreibt und Apoptose (programmierter Zelltod) blockiert. Es wird angenommen, dass die NF-κB Aktivierung eine der möglichen Ursachen der Resistenz von Tumorzellen gegen Chemotherapie oder Bestrahlung sein könnte. Man hat jedoch nicht verstanden, wie NF-κB durch DNA Schäden, die durch die Therapie erzeugt werden, um Tumorzellen durch Apoptose zu beseitigen, aktiviert wird. Im Laboratorium von* ***Claus Scheidereit*** *ist es jetzt gelungen, das Startsignal für das NF-κB abhängige Überlebensprogramm zu identifizieren (publiziert in Molecular Cell): PARP-1, das DNA Strangbrüche innerhalb von Sekunden erkennt, bildet Poly(ADP-Ribose), die als Gerüstmolekül wirkt und IKKγ, die Kinase ATM, sowie weitere Signalmoleküle im Zellkern bindet. Nachfolgende in IKK eingefügte chemische Veränderungen lösen dann NF-κB Aktivierung und damit Schutz gegen Apoptose aus. Diese Ergebnisse sind für klinische Studien wichtig, bei denen PARP-1 Inhibitoren in der Tumortherapie getestet werden.*

In der Embryonalentwicklung wird NF-κB für die komplette Bildung epidermaler Strukturen, einschließlich Haarfollikel, exokriner Drüsen oder von Zähnen benötigt. Durch Verwendung von Mausmodellen konnte jetzt gezeigt werden, wie NF-κB und Wnt/β-Catenin Signalketten bei der Bildung und dem Wachstum der frühen Anlagen dieser Strukturen miteinander interagieren (publiziert in Developmental Cell). Eine Wechselwirkung zwischen den NF-κB und Wnt/β-Catenin Signalketten kann auch in epithelialen Tumoren wichtig sein, bei denen beide aktiviert sind.

Neben dem apoptotischen Zelltod ist Seneszenz als ein weiteres Schutzprogramm erkannt worden, mit dem Zellen auf die Aktivierung von Onkogenen oder Schädigung der DNA antworten können, indem ein irreversibler Wachstumsarrest in der G1 Phase des Zellzyklus ausgelöst wird. Die Gruppe von ***Clemens Schmitt*** *hat eine bahnbrechenden Entdeckung gemacht, indem sie zeigen konnte, dass das Seneszenz-Programm der Tumorenstehung entgegenwirkt und die Induktion von Lymphomen durch das Ras-Onkoprotein verhindern kann. Zu dem zugrundeliegenden Mechanismus wurde herausgefunden, dass ein Enzym beteiligt ist, das bereits dafür bekannt war, Gene*

chromatin. These results identify senescence as a novel tumor suppressor mechanism whose inactivation permits the formation of aggressive but apoptosis-competent lymphomas.

Basic mechanisms in cell biology: from protein quality control to gene boundaries

The controlled degradation of proteins is an important step in cellular regulation processes and homeostasis. Proteins that are damaged by heat, oxidation, or other events become toxic to cells because of their tendency to form aggregates. Protein quality control (PQC) pathways decide whether these faulty polypeptides are channeled into a re-folding pathway or whether they are removed by the ubiquitin-proteasome pathway. PQC pathways are found in many cellular compartments and are essential for proper stress response, development, and cancer prevention. A major PQC pathway is found in the Endoplasmic Reticulum (ER). Dysfunctions in this system lead to severe diseases and in addition, some viruses highjack this system to establish themselves in the infected cell. **Thomas Sommer**'s lab has unraveled the ER-associated protein degradation (ERAD) pathway which is a basic cell biological process that exists in all eukaryotic cells in a highly conserved manner. In the last years, the group could identify the crucial signal that distinguishes a terminally misfolded protein from an unfolded newly synthesized protein. This signal is a specifically processed glycan structure attached to the protein, a Man7-GlcNAc2. This signal is generated by Htm1 and it is decoded by two components of the ERAD specific HRD ubiquitin ligase. The lectin Yos9 binds the oligosaccharide while Hrd3 binds directly to unfolded polypeptides. This dual recognition provides the selectivity in the ERAD pathway.

Investigation of signaling by the Wnt, BMP, and TGFβ cascades in human embryonic stem cells is the focus of research of **Daniel Besser**'s lab. It was found that FGF treated murine fibroblasts produce secreted factors that maintain pluripotency in human stem cells. Identification of these factors by expression profile analysis may help to close the knowledge gap between murine and human embryonal stem cells.

durch Methylierung von Histonen im Chromatin abzuschalten. Durch diese Ergebnisse wurde Seneszenz als ein neuartiger Tumorsuppressions-Mechanismus identifiziert, dessen Inaktivierung die Bildung eines aggressiven, aber zur Apoptose befähigten Lymphoms erlaubt.

Grundlegende zellbiologische Funktionen: Von der Qualitätskontrolle von Proteinen bis zur Abgrenzung von Gen-Orten

*Der kontrollierte Abbau von Proteinen ist ein wichtiger Schritt in zellulären Regulationsprozessen und bei der Homeostase. Proteine, die durch Hitze, Oxidation oder andere Einflüsse geschädigt wurden, haben toxische Auswirkungen, da sie die Tendenz haben, intrazelluläre Aggregate zu bilden. Um mit diesen schädigenden Eigenschaften umzugehen, entscheiden so genannte Protein-Qualitäts-Kontroll (PQC) Systeme, ob die fehlerhaften Proteine repariert werden können oder ob sie der Proteolyse zugeführt werden müssen. Um diese Kontrollfunktion ausführen zu können, kooperiert das Ubiquitin-Proteasom-System mit der zellulären Protein-Faltungsmaschinerie. Solche PQC Systeme kommen in allen zellulären Kompartimenten vor und sind essentiell für störungsfreie Entwicklungsvorgänge und für die Verhinderung von Krebswachstum. Einer der wichtigsten PQC-Pathways befindet sich im Endoplasmatischen Retikulum (ER). Fehlfunktionen dieses Systems können die Ursache schwerer Krankheiten werden. Überdies benutzen einige Viren dieses System, um sich in der infizierten Zelle zu etablieren. Das Laboratorium von **Thomas Sommer** hat die Wirkungsweise des ER-assoziierten Protein-Degradationswegs (ERAD) aufgeklärt, ein grundlegender zellbiologischer Prozess, der in allen eukaryotischen Zellen in evolutionär hoch konservierter Weise vorhanden ist. Im Laufe der letzten Jahre gelang es, das entscheidende Signal zu identifizieren, das ein entgültig fehlgefaltetes Protein von einem ungefalteten neu synthetisierten Protein unterscheidet. Dieses Signal ist eine spezifisch prozessierte Glycan Struktur, die an das Protein geheftet wird, ein Man7-GlcNAc2. Dieses Signal wird durch Htm1 erzeugt und von zwei Komponenten der ERAD-spezifischen Ubiquitinligase erkannt. Das Lectin Yos9 bindet das Oligosaccharid, während Hrd3 direkt an das ungefaltete Polypeptid bindet. Diese duale Erkennung ist für die hohe Spezifität des Prozesses entscheidend.*

*Die Erforschung der Wnt, BMP und TGF-β Signalketten in menschlichen embryonalen Stammzellen steht im Mittelpunkt der Arbeiten von **Daniel Besser**s Gruppe. Es wurde herausgefunden, dass FGF-behandelte Mausfibroblasten*

How Notch- and TGFβ signaling cascades are interconnected is studied by the group of **Harald Saumweber** using the fruit fly as a model organism. Activated Notch signaling targets the CBF1 complex in the nucleus. CBF1 is the core component of a gene regulatory complex that may change from a repressor to an activator. TGFβ signaling activates Smad transcription factors. The Bx42/SKIP protein was found to be involved in both Notch and TGF -dependent gene regulation. Circumstantial evidence suggests that Bx42/SKIP recruits chromatin modifiers and alters gene expression in response to Notch and to TGFβ in an epigenetic fashion. In another project, gene boundaries are studied on Drosophila giant chromosomes. Proper boundary formation between chromosomal genes shield against adjacent genes and heterochromatic gene silencing. Examination of defined loci in different cell types revealed the presence of a protein complex responsible for targeting of several histone modifying enzymes (Jil-1, H3K4-HMT) that are required for local chromatin decondensation and boundary formation between chromatin domains that differ in their degree of condensation.

Structural and Functional Genomics

Structural biologists aim at describing basic aspects of physiological and pathophysiological processes at the level of individual molecules and their interactions. At MDC, two groups employ X-ray crystallography to gain knowledge of the three-dimensional structures of proteins, nucleic acids, membranes, carbohydrates and small metabolites that can provide unique insights into cellular processes and may permit the design of small molecules that specifically modify protein and cellular function.

In the laboratory of **Udo Heinemann**, protein-nucleic-acid interactions, vesicular transport at the Golgi membrane, and cellular processes related to human diseases are being characterized at the atomic level. In a series of crystallographic and biochemical studies, the co-operation of transcriptional regulators in controlling gene expression in the conjugative plasmid RP4 was elucidated, leading to a model according to which a flexible N-terminal domain of the repressor protein KorB binds to a conserved surface of

bestimmte Faktoren sezernieren, die Pluripotenz bei menschlichen Stammzellen erhalten können. Diese Faktoren werden gegenwärtig durch Analyse von Expressionsprofilen identifiziert und sollten später helfen, die Wissenslücke hinsichtlich der Eigenschaften von murinen und humanen embryonalen Stammzellen zu schließen.

*Wie die Notch- und TGFβ-Signalketten miteinander vernetzt sind, wird im Labor von **Harald Saumweber** in der Fruchtfliege als Modellorganismus untersucht. Die aktivierte Notch Signalübertragung ist auf den CBF1-Komplex im Zellkern ausgerichtet. Dieses CBF1 ist die Kernkomponente eines regulatorischen Komplexes, der von einer Gen-Repressorfunktion auf eine Aktivatorfunktion umschalten kann. Durch TGFβ vermittelte Signalübertragungen werden Smad Transkriptionsfaktoren aktiviert. Es wurde herausgefunden, dass das Bx42/SKIP Protein dabei sowohl an Notch- als auch TGFβ-vermittelter Genexpression beteiligt ist. Es liegen Hinweise vor, dass Bx42/SKIP Chromatin-Modifikatoren rekrutiert und dadurch die durch Notch und TGFβ ausgelöste Genexpression auf der epigenetischen Ebene verändert. In einem weiteren Projekt werden die Grenzen zwischen den in Riesenchromosomen in* Drosophila *vorhandenen Genen untersucht. Eine genaue Grenzziehung zwischen chromosomalen Genen schirmt gegen benachbarte Gene und gegen heterochromatinbedingte Stilllegung von Genen, „gene silencing", ab. An den untersuchten genetischen Orten wurde in allen bisher untersuchten Zelltypen die Anreicherung eines Proteinkomplexes gefunden, der verschiedene Histon-modifizierende Aktivitäten hinzuziehen kann (Jil-1, H3K4-HMT); diese sind für eine lokale Öffnung des Chromatins sowie für eine Abgrenzung benachbarter Bereiche unterschiedlich kondensierten Chromatins voneinander notwendig.*

Strukturgenomik und Funktionelle Genomik

Strukturbiologen sind bestrebt, grundlegende Aspekte physiologischer und pathophysiologischer Prozesse auf der Ebene einzelner Moleküle und molekularer Wechselwirkungen zu beschreiben. Zwei Forschungsgruppen des Max-Delbrück-Centrums nutzen die Methode der Röntgenkristallographie, um die dreidimensionalen Strukturen von Proteinen, Nukleinsäuren, Membranen, Kohlenhydraten und Metaboliten zu untersuchen. Diese Strukturen können sehr detaillierte Einsichten in zelluläre Prozesse vermitteln und bei der Identifizierung kleiner Moleküle helfen, welche die Funktion von Proteinen oder Zellen modulieren.

another transcription factor, KorA, at a fixed distance, but with variable geometry. The crystal structure of the Golgi marker protein p115 revealed a dimeric armadillo-repeat structure of its globular head region. This structure could be extended into a model that contains the extended coiled-coil region of p115 and highlights the interactions of this golgin with Rab GTPases and other factors involved in vesicular transport at the Golgi.

Guanine nucleotide binding proteins (G-proteins) are involved in various cellular processes including signal transduction, protein synthesis, sensual perception, and vesicular transport. Large G-proteins of the dynamin superfamily are mechano-chemical enzymes that use the energy of GTP hydrolysis to actively remodel membranes. The group of **Oliver Daumke** aims to elucidate the interaction and reciprocal modulation of membranes and G-proteins using structural, biochemical and cell-biological methods. Their work is focussed on the membrane remodelling EHD family of G-proteins, the GIMAP GTPases which are proposed to regulate apoptosis in cells of the immune system, and the interferon-induced Mx (myxovirus-resistance) proteins, key effector molecules in the innate immune system mediating cellular resistance against pathogens including influenza virus.

Tumor Immunology

Homeostatic chemokines and their receptors are responsible for lymphocyte trafficking between and within secondary lymphoid tissues and thus required for immune surveillance and the establishment of self-tolerance. The group of **Martin Lipp** is particularly interested in the role of homeostatic chemokine receptors in the development and progression of chronic inflammatory diseases associated with lymphoid neogenesis, i. e. the formation of lymphoid tissue-like structures at sites of inflammation. The group demonstrated that the homeostatic chemokine receptors CXCR5 and CCR7 are critical signaling molecules in lymphoid neo-genesis during chronic inflammation. To this end, they have established a novel combined mouse model of collagen- and antigen-induced arthritis showing specific fea-

*Im Labor von **Udo Heinemann** werden Protein-Nukleinsäure-Wechselwirkungen, vesikulärer Transport an der Golgi-Membran und verschiedene krankheitsbezogene zelluläre Prozesse bei atomarer Auflösung untersucht. In einer Reihe kristallographischer und biochemischer Studien wurde die Kooperation von Regulatorproteinen der Transkription des konjugativen Plasmids RP4 untersucht. Diese Arbeiten führten zu einem Modell, nach dem die flexible N-terminale Domäne des Repressorproteins KorB an eine konservierte Oberfläche eines zweiten Transkriptionsfaktors, KorA, über einen festgelegte Abstand, aber mit variabler Geometrie bindet. Die Kristallstruktur des Golgi-Markerproteins p115 zeigte eine dimere aus Armadillo-Motiven aufgebaute Struktur seiner globulären Kopfdomäne. Diese Struktur konnte zu einem Modell komplettiert werden, das die langgestreckte superhelikale Region des p115 enthält und molekulare Wechselwirkungen dieses Golgins mit Rab-GTPasen und anderen Faktoren des vesikulären Transports am Golgi aufzeigt.*

*Guaninnukleotid-bindende Proteine (G-Proteine) sind an verschiedenen zellulären Prozessen einschließlich der intrazellulären Signalübertragung, Proteinsynthese, Sinneswahrnehmung und dem vesikulären Transport beteiligt. Große G-Proteine der Dynamin-Superfamilie sind mechano-chemische Enzyme, die die Energie der GTP-Hydrolyse nutzen, um Membranen aktiv zu remodellieren. Die Gruppe von **Oliver Daumke** studiert die Wechselwirkung und wechselseitige Modulierung von Membranen und G-Proteinen mit strukturellen, biochemischen und zellbiologischen Methoden. Ihre Arbeiten sind auf die EHD-Familie von Membran-remodellierenden G-Proteinen, die GIMAP-GTPasen, denen eine Funktion bei der Regulierung der Apoptose in Zellen des Immunsystems zugeschrieben wird, sowie auf die Interferon-induzierten Mx- (Myxovirus-resistenten) Proteine, die als wichtige Effektoren des angeborenen Immunsystems die zelluläre Abwehr von Pathogenen wie dem Grippevirus vermitteln, fokussiert.*

Tumorimmunologie

*Die Migration von Lymphozyten zwischen und innerhalb von lymphoiden Geweben hängt von homöostatischen Chemokinen und deren Rezeptoren ab, die deshalb für die Immunüberwachung und die Ausbildung von Autoimmuntoleranz notwendig sind. Die Gruppe von **Martin Lipp** ist in besonderem Maße an der Rolle der homöostatischen Chemokine bei der Entstehung und Progression chronischer Entzündungen interessiert, die mit lymphoider Neogenese einhergehen, d.h. mit der Bildung lymphknoten-*

tures of the chronic phase in human disease. In addition, the model is characterized by formation of antibodies directed against collagen type II and citrullinated peptides (CCP). CCP-specific antibodies have a high prognostic value in humans and have so far not been described in mouse models of arthritis. Another recent accomplishment of the group was to show that CCR7 is also a key regulator of homeostatic lymphocyte recirculation through body cavities and non-lymphoid peripheral tissues.

The adoptive transfer of regulatory Foxp3-positive T cells (Treg) has been shown in various animal models to prevent inflammatory immune and autoimmune diseases. Translation into therapeutic applications, however, is hindered by the lack of suitable techniques and markers. CD25, commonly used to isolate Treg cells from mice, has only limited value in humans as it is also present on proinflammatory CD4-positive effector cells. The group of **Olaf Rötzschke** and **Kirsten Falk** has shown that pure populations of human Treg cells can be obtained with antibodies directed against the integrin alpha subunit CD49d. The marker is present on proinflammatory peripheral blood mononuclear cells but is absent on immune-suppressive Treg cells. Importantly, in combination with the marker alpha-CD127 it allows the isolation of "untouched" Treg cells, that have not been targeted by an antibody during purification, and that are virtually free of contaminating CD25-positive effector cells. The cells can be expanded in vitro and are effective suppressors both in vitro and in vivo. Thus, CD49d provides access to highly pure populations of untouched Treg cells conferring maximal safety for future clinical applications.

The immune system usually fails to reject established tumors. In order to develop effective cancer therapies, it is therefore essential to understand the molecular interaction of tumor cells, immune cells, and the tumor microenvironment. The group of **Thomas Blankenstein** has shown in a model of sporadic immunogenic cancer, that tumor-specific tolerance closely coincides with the first tumor antigen recognition by B cells. During the subsequent latency period until tumors progress, the mice acquire general cytotoxic T lymphocyte (CTL) unresponsiveness. In

ähnlicher Gewebsstrukturen in der Umgebung von Entzündungsherden. Die Gruppe konnte nachweisen, dass die Chemokin-Rezeptoren CXCR5 und CCR7 für die Signalübertragung bei der lymphoiden Neogenese im Verlaufe chronischer Entzündungen kritische Moleküle darstellen. Für diese Studien wurde ein neuartiges kombiniertes Mausmodell Kollagen-und Antigen-induzierter Arthritis etabliert, das spezifische Merkmale der chronischen Phase der Erkrankung beim Menschen aufweist. Dieses Modell ist weiterhin dadurch ausgezeichnet, dass Antikörper gegen Kollagen Typ II und citrullinierte Peptide (CCP) gebildet werden. CCP-spezifische Antikörper haben eine hohe prognostische Bedeutung beim Menschen und sind vorher noch nicht für Arthritismodelle der Maus beschrieben worden. Eine weiterer kürzlicher Erfolg der Arbeitsgruppe war der Nachweis, dass CCR7 auch einen Schlüsselregulator der homöostatischen Lymphozyten-Rezirkulation durch Körperhöhlen und nicht-lymphoide periphere Gewebe darstellt.

Es ist in verschiedenen Tiermodellen gezeigt worden, dass der adoptive Transfer von regulatorischen FoxP3-positiven T Zellen (Treg) entzündliche Immun- und Autoimmunerkrankungen verhindern kann. Die Umsetzung in therapeutische Anwendungen ist jedoch wegen fehlender Techniken und Marker erschwert worden. CD25, welches häufig verwendet wird, um Treg aus Mäusen zu isolieren, hat beim Menschen einen nur geringen Nutzen, da es dort ebenfalls auf pro-entzündlichen CD4-positiven Effektor T Zellen vorhanden ist. Die Arbeitsgruppe von ***Olaf Rötzschke*** *und* ***Kirsten Falk*** *hat herausgefunden, dass reine Populationen humaner Treg mit Antikörpern erhalten werden können, die gegen die Integrin α Untereinheit CD49d gerichtet sind. Dieser Marker ist auf pro-entzündlichen peripheren mononukleären Blutzellen vorhanden, aber nicht auf immun-suppressiven Trg Zellen. In Kombination mit dem Marker α-CD127 können damit „unberührte" Treg Zellen isoliert werden, die während der Reinigung noch nicht mit einem Antikörper in Berührung gekommen sind und die vollkommen frei von kontaminierenden CD25-positiven Zellen sind. Die Zellen können in* vitro *vermehrt werden und sind wirkungsvolle Suppressoren sowohl* in vitro *als auch* in vivo. *Damit liefert CD49d Zugang zu hochgradig reinen Populationen unberührter Treg Zellen, womit maximale Sicherheit für künftige klinische Anwendungen gewährleistet wird.*

Eine Abstoßung etablierter Tumoren wird durch das Immunsystem meist nicht zustande gebracht. Für die Entwicklung wirksamer Behandlungsverfahren gegen Krebs

mice with large non-immunogenic tumors, unrelated CTL responses are undiminished. These results suggest that (a) tolerance to the tumor antigen occurs at the premalignant stage, (b) tumor latency is unlikely caused by CTL control, and (c) a persistent immunogenic tumor antigen causes general CTL unresponsiveness but tumor burden per se does not.

Cytotoxic T cells destroy virus-infected and tumorigenic cells through release (exocytosis) of cytotoxic substances from lytic granules. In recent years scientists have identified molecules within CTLs that help secrete the toxins. But little is known about the contrary process, which keeps levels of the substances low and prevents their release at the wrong time. The laboratories of **Armin Rehm**, **Uta Höpken**, and **Bernd Dörken** at the MDC and Charité University Hospital, along with several collaborating groups, have explored a new mechanism that helps cells control the toxins. By generating and analyzing Ebag9 (estrogen receptor-binding fragment-associated antigen 9)-deficient mice they showed that loss of EBAG9 confers CTLs with enhanced cytolytic capacity in vitro and in vivo. Although loss of EBAG9 did not affect lymphocyte development, it led to an increase in CTL secretion of granzyme A, a marker of lytic granules. This resulted in increased cytotoxicity *in vitro* and an enhanced cytolytic primary and memory T cell response *in vivo*. While EBAG9 deficiency did not disrupt the formation of the immunological synapse, lytic granules in Ebag9-deficient CTLs were smaller than in wild-type CTLs. These results identify a potential link between the hormone estrogen and the way the immune system responds to cancer and suggest that EBAG9, which seems to be abnormally expressed in some human tumors, is a tunable inhibitor of CTL-mediated adaptive immune response functions.

The groups of **Thomas Blankenstein**, **Wolfgang Uckert** and **Antonio Pezzutto** have a strong interest in cancer immunotherapy based on adoptive T cell transfer. In a collaborative effort they have developed novel methods to generate T cells expressing a recombinant T cell receptor (TCR) with defined antigen specificity. TCR gene therapy bears the risk of auto-reactive side-effects when the TCR recognizes antigens on self-tissue. The groups have now devel-

ist es daher von Bedeutung, die molekularen Interaktionen von Tumorzellen mit Immunzellen und mit ihrer Mikroumgebung zu verstehen. Die Gruppe von ***Thomas Blankenstein*** *hat mit einem sporadischen immunogenen Krebsmodell zeigen können, dass eine Tumor-spezifische Toleranz dicht mit der ersten Tumorantigen-Erkennung durch B Zellen zusammenfällt. Während der nachfolgenden Latenzperiode bis sich die Tumoren weiterentwickeln, erwerben die Mäuse eine allgemeine Unansprechbarkeit der zytotoxischen T Lymphozyten (CTL). In Mäusen mit großen, nicht-immunogenen Tumoren sind hierauf nicht bezogene CTL Antworten unbeeinträchtigt. Diese Ergebnisse legen nahe, dass (a) eine Toleranz gegen das Tumorantigen im prämalignen Stadium auftritt, (b) die Tumorlatenz wohl kaum durch CTL verursacht wird und (c), dass ein fortbestehendes immunogenes Tumorantigen eine allgemeine CTL Unansprechbarkeit verursacht, während dies durch die Tumorlast allein nicht erreicht wird.*

Zytotoxische T Zellen zerstören Virus-infizierte und tumorigene Zellen durch Freisetzung (Exozytose) von zytotoxischen Substanzen aus lytischen Körnern (lytic granules). In den vergangenen Jahren haben Wissenschaftler Moleküle innerhalb der CTL identifiziert, die bei der Ausschüttung von Toxinen behilflich sind. Es war aber wenig über den gegenläufigen Prozess bekannt, der die Menge dieser Substanzen niedrig hält und verhindert, dass deren Freisetzung zum falschen Zeitpunkt stattfindet. Die Laboratorien von ***Armin Rehm***, ***Uta Höpken*** *und* ***Bernd Dörken*** *am MDC und an der Charité Universitätsklinik haben, zusammen mit mehreren kollaborierenden Arbeitsgruppen, einen neuen Mechanismus ergründet, mit dem Zellen Toxine unter Kontrolle halten. Durch die Generierung and Analyse* Ebag9 *(estrogen receptor-binding fragment associated antigen 9)-defizienter Mäuse konnten sie zeigen, dass CTL durch den EBAG9 Verlust eine verstärkte zytolytische Aktivität, sowohl* in vitro *als auch* in vivo*, erworben haben. Obgleich der EBAG9 Verlust keinen Einfluss auf die Entwicklung der Lymphozyten hatte, führte er zu einer Verstärkung der Auschüttung von Granzym A, einem Marker der lytischen Körner. Dies führte* in vitro *zu einer erhöhten Zytotoxizität und* in vivo *zu einer verstärkten Antwort der zytolytischen primären und Gedächtnis (memory) T Zellen. Während der EBAG9 Verlust nicht zu einem Zerfall der immunologischen Synapse führte, waren die lytischen Körner in EBAG9-defizienten CTL kleiner als in normalen CTL. Durch diese Ergebnisse konnte eine mögliche Verbindung zwischen dem Hormon Östrogen und der Weise, wie das Immunsystem auf Krebs reagiert, hergestellt werden und*

oped a new safeguard that is based on a TCR-intrinsic depletion mechanism to eliminate auto-reactive TCR-redirected T cells. By modifying TCRs with a 10 amino acid tag of the c-myc protein, which does not interfere with TCR-mediated effector function, T cells harbouring tag-modified TCRs can be efficiently depleted *in vivo* with a tag-specific antibody. More important, *in vivo* depletion of adoptively transferred T cells rescued mice from lethal autoimmune diabetes. This new safeguard will allow the termination of adoptive therapy in case of severe side-effects.

es wird nahegelegt, dass EBAG9, welches in einigen Tumoren in abweichender Weise exprimiert wird, ein veränderbarer Hemmstoff CTL-vermittelter adaptiver Immunfunktionen ist.

Die Arbeitsgruppen von ***Thomas Blankenstein****,* ***Wolfgang Uckert*** *und* ***Antonio Pezzutto*** *haben ein starkes Interesse an Krebs-Immunotherapie, die auf adoptiven Transfer von T Zellen basiert ist. In einer kollaborativen Initiative wurden neue Methoden entwickelt, um T Zellen zu generieren, die einen rekombinanten T Zellrezeptor (TCR) mit definierter Antigen-Spezifität exprimieren. TCR-Gentherapie trägt das Risiko von auto-reaktiven Nebeneffekten, wenn der TCR Antigene auf Eigengewebe erkennt. Die Arbeitsgruppen haben jetzt eine neues Sicherungssystem entwickelt, das auf einem TCR-gerichteten Depletierungsmechanismus beruht, um auto-reaktive TCR-modifizierte T Zellen zu eliminieren. Durch Anheftung einer 10 Aminosäuren langen Sequenz des c-Myc Proteins an den TCR, wodurch die TCR-Funktion nicht gestört wird, können T Zellen, die diese Sequenz tragen,* in vivo *effizient mit einem spezifischen Antikörper depletiert werden. Wichtig ist, dass beobachtet werden konnte, dass eine* in vivo *Depletierung übertragener T Zellen Mäuse vor tödlicher Autoimmun-Diabetes retten konnte. Dieses neue Sicherungssystem wird es erlauben, eine adoptive Therapie im Falle schwerer Nebeneffekte abzubrechen.*

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Signal Transduction in Tumor Cells

The major interest of our laboratory is the regulation of gene expression by cellular signal transduction processes. "Nuclear factor kappaB" (NF-κB) denotes a family of transcription factors whose activities are controlled by inhibitory IκB proteins and IκB kinases (IKK). NF-κB/IKK signaling cascades have wide physiological and medical relevance. Major efforts are to decipher the mechanisms and structures that underlie gene regulation by IKK and NF-κB, the crosstalk with other gene regulatory systems and to dissect both, the role in development and in the pathogenesis of diseases.

Molecules and mechanisms that control IKK and NF-κB activity

Most if not all cell types of the body utilize NF-κB transcription factors to regulate under various conditions the expression of numerous genes. These encode cytokines, surface receptors, adhesion molecules, transcription factors and other functional classes of proteins. Biological processes which involve NF-κB activation include the innate and adaptive immune responses, inflammation, cellular reaction to environmental stress as well as selective aspects of early embryonic development. In non-stimulated cells, NF-κB is associated with IκB molecules, which inhibit nuclear translocation and DNA binding activity of NF-κB. Cellular exposure to a variety of agents or conditions, including microbial pathogens, cytokines, mitogens, genotoxic stress or morphogens triggers the activation of an IκB kinase (IKK) complex, which consists of catalytic (IKKα, IKKβ) and regulatory (IKKγ/NEMO) components. This complex phosphorylates IκB molecules, resulting in their ubiquitination by βTrCP/SCF ubiquitin ligases and proteasomal destruction, followed by liberation of active NF-κB.

In mammals, the NF-κB family comprises five related members, p50, p52, p65, c-Rel and RelB. These proteins form distinct hetero- and homodimers and bind to inhibitory cytoplasmic IκB molecules, IκB α,β or ε, or to the nuclear IκB homologues Bcl-3 and MAIL. It is a characteristic feature of NF-κB that two of its subunits, p50 and p52, are formed by proteolytic proteasomal processing of their precursor proteins, p105 and p100, respectively. Unprocessed p105 and p100 bind to other NF-κB subunits and so act as cytoplasmic inhibitors.

There are two distinct IKK pathways, which respond to extracellular stimuli and either trigger degradation of IκBs and liberation of prototypic p50-p65 (canonical pathway) or proteolytic processing of p100 and production of p52-RelB complexes (non-canonical pathway) (Figure 1). The two pathways depend on IKKβ and IKKγ/NEMO or on IKKα, respectively. For the canonical pathway, a critical role for non-degradative, K63-linked poly-ubiquitination has been recognized. These ubiquitin modifications are generated by ubiquitin ligases, such as TRAF2 or TRAF6, and enforce a co-recruitment of the IKK complex and other components, which have ubiquitin binding motifs, to receptor-proximal complexes. This results in T-loop phosphorylation and activation of IKKs. A third IKK pathway is activated by DNA damage-inducing agents or conditions, such as γ-irradiation or chemotherapeutic drugs in the nucleus and

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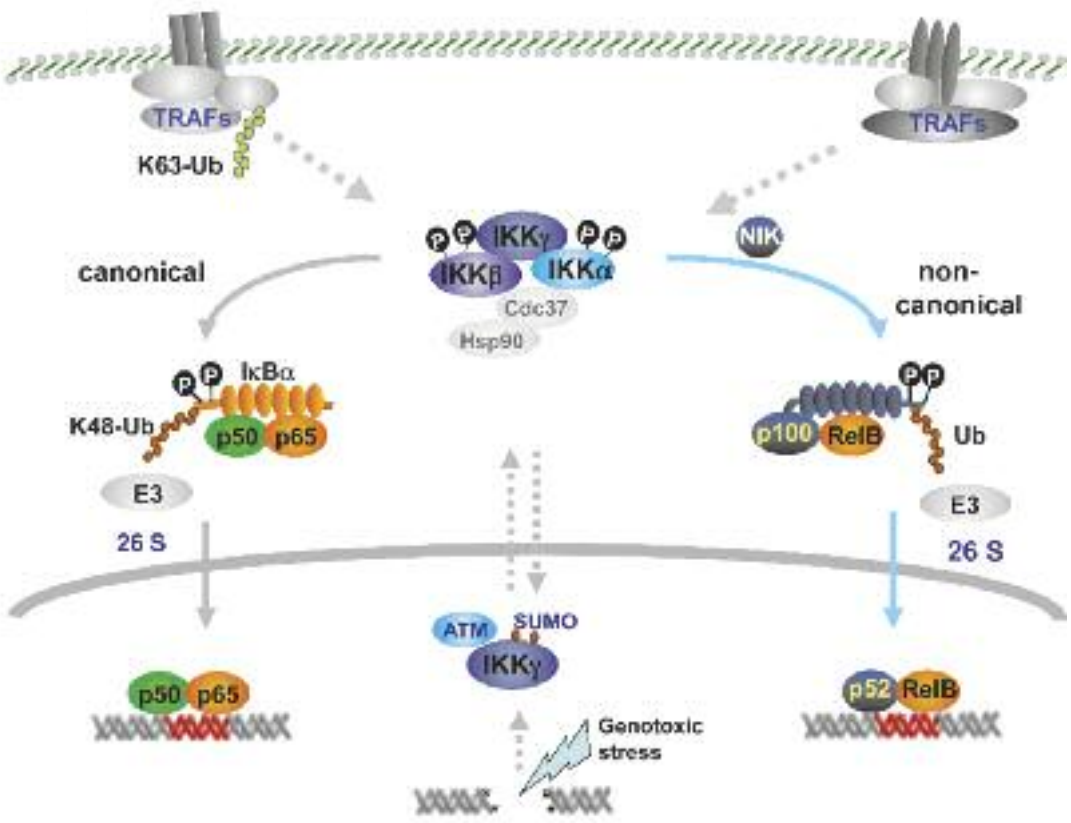

Figure 1. *Distinct NF-κB dimers are activated by canonical and non-canonical IKK/NF-κB signaling pathways and depend on IKKβ and IKKα, respectively. Both pathways are constitutively activated in Hodgkin lymphoma cells. A nucleus-to-cytoplasm IKK/NF-κB pathway is induced by the DNA damage response and may play a role in therapy resistance of tumor cells. Homeostasis and activity of the IKK complex is regulated by the Hsp90-Cdc37 chaperone complex.*

depends on nuclear shuttling and SUMO-modification of IKKγ, as well as on the kinase ATM.

IKK and NF-κB signal transduction in lymphoid tumor cells

A dysregulation of the IKK/NF-κB system has now been recognized as an oncogenic hallmark of an increasing number of lymphoma and leukemia entities. We have investigated the origins and the biological functions of constitutively activated IKKs and NF-κB and the downstream effector networks in Hodgkin lymphoma (HL) tumor cells. The IKK/NF-κB system is aberrantly activated in a cell-autonomous manner, generally involving a persistent activation of the IKK complex, which results in constitutive release of NF-κB complexes. In HL cells, both, canonical and non-canonical p100 pathways are activated (Figure 1). The determination of the global NF-κB target gene signature in HL cells by microarrays

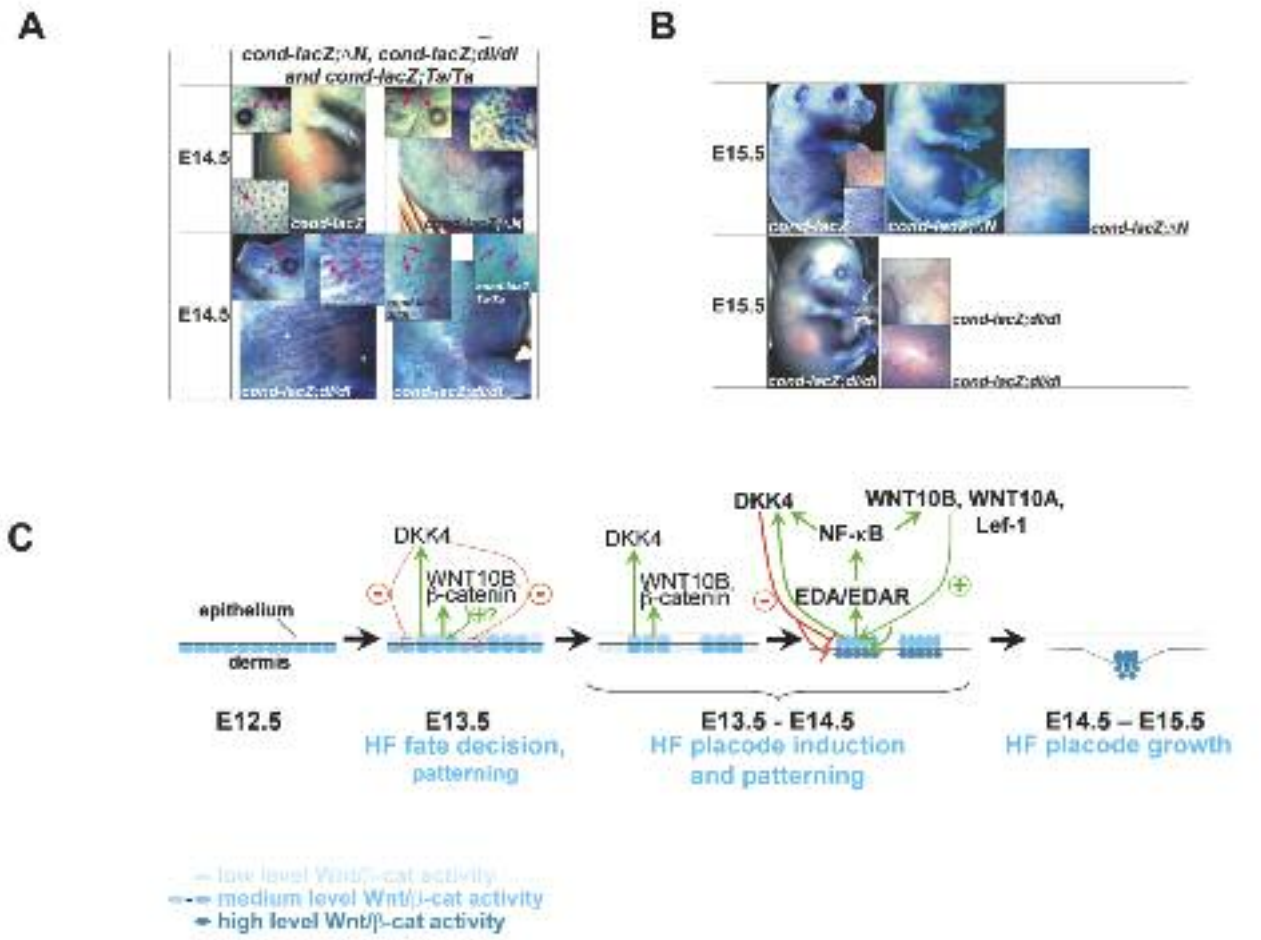

Figure 2. *Mutual requirements for Eda-A1/Edar/NF-κB and Wnt/β-catenin in early hair follicle development. (A) Wnt activity is maintained in the absence of Eda-A1/Edar/NF-κB activity. Wnt reporter mice (cond-lacZ) were mated with mice deficient in Eda-A1 (cond-lacZ;Ta/Ta) or Edar expression (cond-lacZ;dl/dl), or with mice lacking NF-κB activity (cond-lacZ;ΔN). X-Gal stained E14.5 embryos are depicted. Arrows point to Wnt activity in placodes and other ectodermal appendages, such as whiskers and eyelids. Note that the placode pattern in embryos lacking epidermal Eda-A1/Edar/NF-κB activity is irregular when compared to controls. Thus, placode pattern refinement requires NF-κB activity. We and others have shown that Wnt inhibitor Dkk4 is an NF-κB target gene. Dkk4 is known to be involved in placode patterning and spacing. (**B**) X-Gal stained cond-lacZ;dl/dl and cond-lacZ;ΔN embryos at E15.5, when hair placodes have already started to grow. Focal Wnt activity in hair placodes has disappeared in the absence of Eda-A1/Edar/NF-κB activity. We have found that Wnt10b is a direct target gene of Eda-A1/Edar/NF-κB, providing a possible explanation for this observation. Panel (**C**) illustrates a model for the molecular interplay of Wnt/β-catenin and Edar/NF-κB signaling pathways. Note that Wnt10a and Lef-1 have previously been described as potential NF-κB target genes.*

revealed that constitutive NF-κB drives the expression of genes with important functions in cell cycle progression, programmed cell death and tropism or migration of tumor cells. Thus, a central pathogenic role of the IKK/NF-κB pathways is very likely and the distinct contributions of canonical and non-canonical pathways for the biology of the tumor cells are now under investigation.

In addition to IKK/NF-κB, Hodgkin lymphoma cells reveal aberrant activation of transcription factor AP-1 (activating protein 1), composed of the c-Jun and JunB subunits. AP-1 cooperates with NF-κB to superactivate a subset of NF-κB target genes in HL. Furthermore, Stat5a is activated by NF-κB in HL cells and may synergize with NF-κB at the level of common target genes.

By a genome-wide determination of genes regulated by IKK and NF-κB in activated B cell lineage cells, we could show that induced AP-1 activity is entirely dependent on IKK and NF-κB, which regulate expression of Jun, ATF and Maf members. This cross-talk is under further

investigation, as is the cause of constitutive IKK/NF-κB and c-Jun activation in Hodgkin lymphoma.

Functional control of IKK by transient interaction with Hsp90 and Cdc37

The IKK complex undergoes interactions with a number of regulatory proteins, including the chaperones Cdc37 and Hsp90. We had previously shown that pharmacological inhibition of Hsp90 abrogates constitutive IKK activation in Hodgkin lymphoma cells, resulting in strongly enhanced apoptosis. Using an RNAi approach, we found that Cdc37 recruits Hsp90 to the IKK complex in a transitory manner, preferentially via IKKα. Binding is conferred by N-terminal as well as C-terminal residues of Cdc37 and results in the phosphorylation of Cdc37. Cdc37 is essential for the maturation of *de novo* synthesized IKKs into enzymatically competent kinases, but not for assembly of an IKK holocomplex. Mature IKKs, T-loop phosphorylated after stimulation either by receptor-mediated signaling or upon DNA damage, further require Hsp90-Cdc37 to generate an enzymatically activated state. Thus, the Hsp90-Cdc37 chaperone-co-chaperone complex is an essential regulatory component in IKK signaling cascades and a potential drug target in tumor therapy.

Mutual requirements of Eda-A1/Edar/NF-κB and Wnt/β-catenin signaling in early ectodermal organogenesis

To allow a functional analysis of NF-κB in early embryonic development and in disease models, we have generated NF-κB repressor and reporter mice. The repressor mice carry a dominant negative IκBα mutant (IκBαΔN) as a conditional knock-in allele, while the reporter mice express an NF-κB-driven β-gal transgene. Using these mice, we could previously demonstrate novel morphogenic functions for NF-κB, including an early role in the development of epidermal organs, such as hair follicles (HF). In early HF placodes, NF-κB is specifically activated by TNF family members Eda-A1 and its receptor Edar. Furthermore we have identified an intense molecular cross-talk of Eda-A1/Edar/NF-κB with other signaling pathways required for HF development, such as Wnt and Sonic Hedgehog (SHH).

According to our current knowledge, Wnt/β-catenin signaling specifies the initial hair fate decision of epidermal keratinocytes, while NF-κB is required for hair placode growth by activating SHH signaling and repressing Bone Morphogenic Protein (BMP) activity. In collaboration with the laboratory of Dr. Sarah E. Millar (U. Pennsylvania, Philadeplhia, USA) we have now demonstrated that Wnt/β-catenin is initially activated independently of Eda-A1/Edar/NF-κB, but depends on NF-κB activity for focal hair placode patterning. In contrast, initial Eda-A1/Edar/NF-κB signaling is not activated in skin of mice expressing the secreted Wnt inhibitor DKK1, or lacking epithelial β-catenin (Figure 2). In this context we have shown that Wnt/β-catenin is absolutely essential for NF-κB activation and have provided evidence that Edar is a direct Wnt/β-catenin target gene. However, at later time points of hair follicle development, localized Wnt activity disappears from Eda-A1/Edar/NF-κB mutant skin, implying that Eda-A1/Edar/NF-κB is required for the maintenance of Wnt signaling (Figure 2). We found that NF-κB is essential for placodal up-regulation of Wnt10a and Lef-1, and we could identify Wnt10b as a direct target gene of NF-κB. Our data reveal a complex interplay and interdependence of Wnt/β-catenin and Eda-A1/Edar/NF-κB signaling pathways, which may not only have important implications in organ development and morphogenesis, but also in cancer and inflammatory diseases.

Our current and future studies will continue to focus on the Wnt/NF-κB cross-talk in development/morphogenesis and disease. Furthermore, in collaboration with Dr. Nathaniel Heintz (Rockefeller I., New York, NY, USA) and Ines Ibañez-Tallon (MDC) we have developed a mouse model (*bacTRAP*: *translating ribosome affinity purification* (TRAP)) that allows the specific isolation of HF keratinocytes. This mouse model will be used to identify novel NF-κB target genes in early HF development and to perform detailed gene profiling studies on the molecular controls of HF induction.

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Stilmann, M, Hinz, M, Cöl Arslan, S, Zimmer, A, Schreiber, V, Scheidereit, C, (2009). A nuclear poly(ADP-ribose) dependent signalosome confers DNA damage induced IκB kinase activation. Mol Cell, in press.

Walter Birchmeier

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Signals Provided by Wnt/β-catenin and Met/Gab1/Shp2 in Development and Cancer

The molecular and functional analysis of cell adhesion and signaling in development and tumor progression has been the major focus of my laboratory. We previously defined functions of E-cadherin/β-catenin, which mediate cell-cell adhesion of epithelial cells and prevent invasion and metastasis. Moreover, we discovered that β-catenin, which is a central component of canonical Wnt signaling (armadillo in Drosophila), binds to the transcription factors LEF/TCF. This interaction allows the nuclear translocation of β-catenin, and the regulation of gene expression. Signals provided by Wnt/β-catenin are essential in many developmental processes and in tumor progression. We have used conditional loss-of-and-gain-of-function mutations to study the role of β-catenin in the skin, and in development of limbs and brain, and observed essential functions of β-catenin signaling in these organs. In the skin, specification of stem cells to the hair, but not the epidermal lineage requires β-catenin signals. In the dorsal spinal cord, Wnt/β-catenin signaling controls the transcription factor Olig3, which is required for the specification of two types of neurons, dI2 and dI2. Moreover, our genetic analysis of two other members of the armadillo gene family, plakoglobin (γ-catenin) and plakophilin2, established a role of theses molecules in the stability of cell junctions in the heart, and implicate these molecules in heart disease. We recently discovered that the β-catenin-binding protein BCL9-2, a homologue of legless in Drosophila, induces epithelial-mesenchymal transition and activation of canonical Wnt signaling.

In addition, we have a long standing interest in the biochemistry of scatter factor/hepatocyte growth factor (SF/HGF) and Met receptor signaling, and have analyzed functions of these molecules in development and tumor progression. We characterized the downstream effectors of Met, Gab1 and Shp2. We were able to show, by genetic means, that signaling of Gab1 through the tyrosine phosphatase Shp2 controls migration of muscle precursor cells to the limbs. Shp2 acts also downstream of many other receptor and non-receptor kinases.

In the present report period, we have performed the following investigations on Wnt/β-catenin and receptor tyrosine kinase signaling:

A colorectal cancer expression profile that includes the β-catenin target and TGFβ inhibitor BAMBI predicts metastatic potential

Johannes Fritzmann, Daniel Besser, Frauke Kosel, Felix H. Brembeck, Ulrike Stein, Iduna Fichtner, Peter M. Schlag, Walter Birchmeier, Markus Morkel (MPI for Molecular Genetics, Berlin) and Jan Budczies (Charité Berlin)

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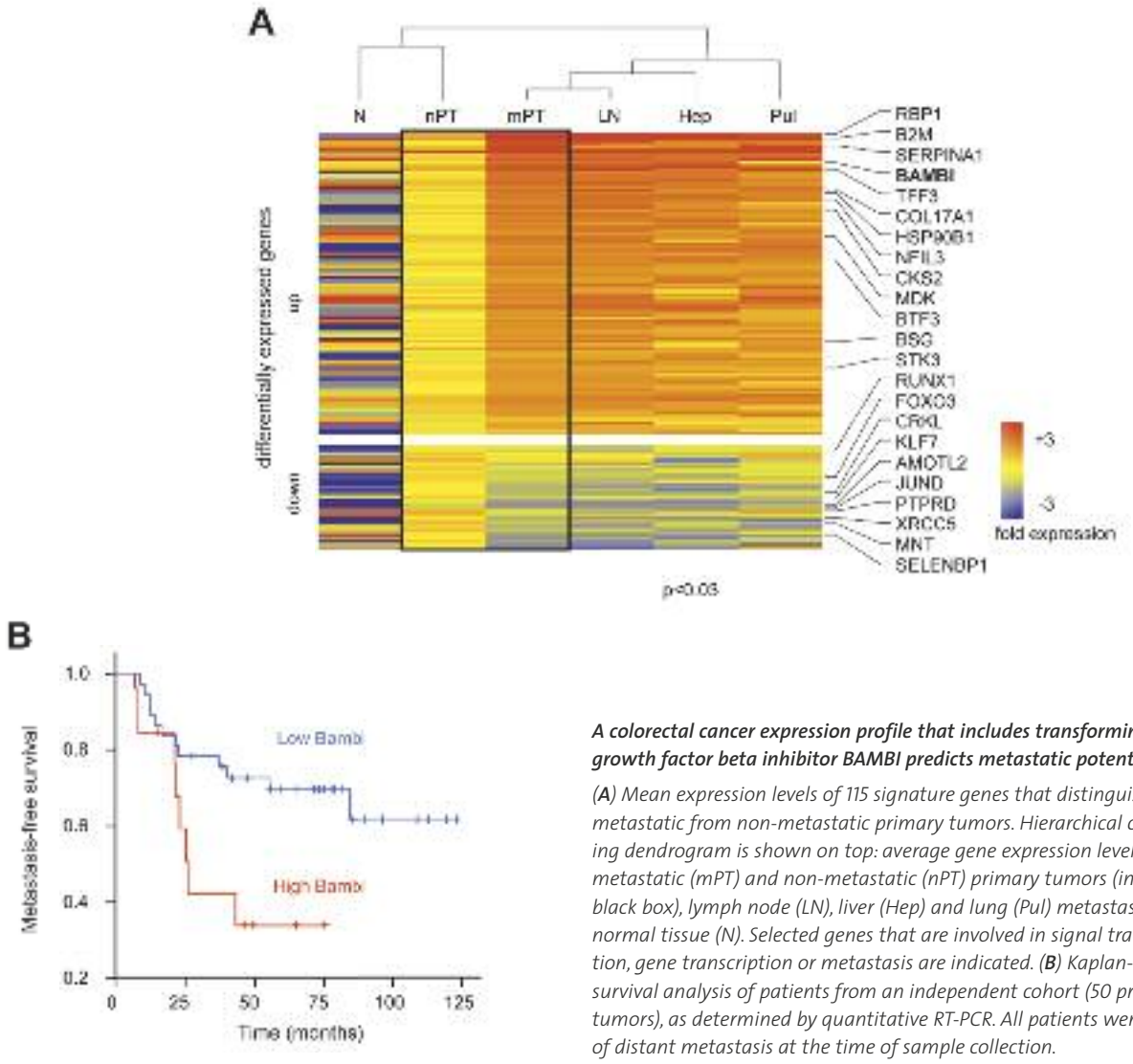
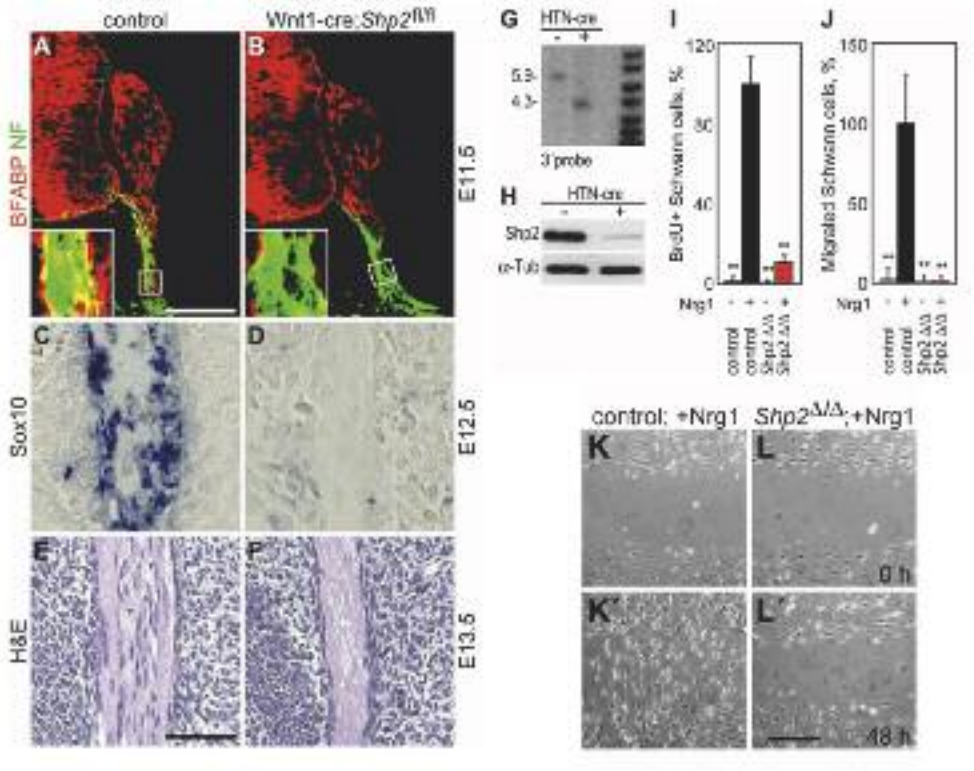


A colorectal cancer expression profile that includes transforming-growth factor beta inhibitor BAMBI predicts metastatic potential

*(**A**) Mean expression levels of 115 signature genes that distinguish metastatic from non-metastatic primary tumors. Hierarchical clustering dendrogram is shown on top: average gene expression levels in metastatic (mPT) and non-metastatic (nPT) primary tumors (inside black box), lymph node (LN), liver (Hep) and lung (Pul) metastases and normal tissue (N). Selected genes that are involved in signal transduction, gene transcription or metastasis are indicated. (**B**) Kaplan-Meier survival analysis of patients from an independent cohort (50 primary tumors), as determined by quantitative RT-PCR. All patients were free of distant metastasis at the time of sample collection.*

Much is known about the genes and mutations that cause colorectal cancer (CRC), yet only a few have been associated with CRC metastasis. Mutations of genes of the Wnt/β-catenin pathway are responsible for the generation of over 90 % of CRC. We performed expression profiling experiments to identify genetic markers of risk and to elucidate the molecular mechanisms of CRC metastasis. We compared gene expression patterns between metastatic and non-metastatic, stage-matched human colorectal carcinomas. We established a signature of 115 genes that differentiated metastatic from non-metastatic primary tumors. Among these, the TGFβ inhibitor BAMBI was highly expressed in about half of metastatic primary tumors and metastases but not in non-metastatic tumors. We have identified BAMBI as a target of canonical Wnt signaling that involves the β-catenin co-activator BCL9-2. We observed an inverse correlation between level of BAMBI expres-



The tyrosine phosphatase Shp2 directs Neuregulin-1/ErbB signaling throughout Schwann cell development

In vivo analysis of Schwann cells associated with peripheral nerves in control and Wnt1-cre Shp2$^{fl/fl}$ mice at the indicated developmental stages using (A,B) immunohistochemistry for BFABP (red) and neurofilament (NF, green), (C,D) in situ hybridization with a Sox10-specific probe, and (E,F) histological sections stained with hematoxylin and eosin. Note the reduced numbers of Schwann cells at Shp2 mutant peripheral nerves.

In vitro analysis of the role of Shp2 during Schwann cell proliferation and migration. (G) Southern blot and (H) Western blot analyses after HTN-cre induced recombination in cultured Shp2$^{fl/fl}$ Schwann cells. (I,J) Quantification of the Nrg1-induced proliferation and migration indicates strong reduction of both cellular responses in the absence of Shp2. Shown are the percentage of Schwann cells that incorporated BrdU, or the percentage of Schwann cells that migrated into the scratched area, using control and Shp2$^{\Delta/\Delta}$ Schwann cells (HTN-cre-treated Shp2$^{fl/fl}$ Schwann cells) in the presence and absence of Nrg1. (K-L´) Migration of Schwann cells into a scratched area.

sion and metastasis-free survival time of patients. BAMBI inhibits TGFβ signaling and increases migration in colon cancer cells. In mice, overexpression of BAMBI caused colon cancer cells to form tumors that metastasized more frequently to liver and lymph nodes than control cancer cells. Thus BAMBI regulates colorectal cancer metastasis by connecting the Wnt/β-catenin and TGFβ signaling pathways. The metastatic expression signature we describe, along with BAMBI levels, may be used in prognosis in the future. Our data also show that developmental signaling pathways act in hierarchies and cooperate in tumor cell migration, invasion and metastasis.

Skin cancer stem cell maintenance is dependant on catenin signaling throughout Schwann cell development

Ilaria Malanchi, Deepika Kassen, Thomas Hussenet, Jörg Huelsken (EPFL/ISREC Lausanne), Amparo Cano (CSIC-UAM Madrid) and Walter Birchmeier.

Proper canonical Wnt signaling guides healthy development in many tissues, but defects play a role in tumors, as revealed by the analysis of loss- and gain-of function mutations in β-catenin. The recently identified cancer stem cells and normal stem cells share many characteristics, like the capacity for self-renewal and differentiation and their dependence on a particular microenvi-

ronment, the (cancer) stem cell niche. We have identified a population of cancer stem cells in mouse epidermal tumors, i.e. early squamous cell carcinomas, which are dependent on β-catenin and that are phenotypically and functionally similar to normal bulge skin stem cells. These cancer stem cells can be isolated by cell sorting based on the presence of the stem cell marker CD34 and absence of other markers. In normal mouse skin, $CD34^+$ bulge stem cells account for approximately 1.8% of keratinocytes. However, cutaneous tumors, induced by chemical (DMBA/TPA) carcinogenesis or by expression of the Ras oncogene, contain a 9-fold increase of the $CD34^+$ cell population. The tumorigenic capacity of the $CD34^+$ cells was over 100-fold greater than that of unsorted cells, when these were transplanted into the back skin of NOD/SCID mice. Secondary tumors that formed after transplantation resembled the parental tumors; they contained a small population of $CD34^+$ stem cells. Remarkably, the deletion of β-catenin in DMBA/TPA or Ras-induced tumors using an inducible system that allows conditional mutagenesis after the addition of tamoxifen ($K14\text{-}cre^{ERT2}$; $\beta\text{-}catenin^{flox}$) resulted in complete tumor regression. Thus, canonical Wnt signals are required for the maintenance of skin cancer stem cells, whereas in normal skin they instruct bulge stem cells towards the hair cell fate.

The tyrosine phosphatase Shp2 directs Neuregulin-1/ErbB signaling throughout Schwann cell development

Katja Grossmann, Hagen Wende, Florian Paul, Cyril Cheret, Alistair Garratt, Daniel Besser, Herbert Schulz, Matthias Selbach, Walter Birchmeier and Carmen Birchmeier.

The non-receptor tyrosine phosphatase Shp2 has been implicated in tyrosine kinase, chemokine and integrin receptor signaling. We have produced a floxed allele of the Shp2 gene in mice in order to study the biological function of this tyrosine phosphatase. We showed that conditional mutation of Shp2 in neural crest cells and in myelinating Schwann cells resulted in deficits in glial development that are remarkably similar to those observed in mice mutant for Neuregulin-1 (Nrg1) or the Nrg1 receptors, ErbB2 and ErbB3. In cultured Shp2 mutant Schwann cells, Nrg1-evoked cellular responses like proliferation and migration were virtually abolished, and Nrg1-dependent intracellular signaling was altered. Pharmacological inhibition of Src family and MAP kinases mimicked all cellular and biochemical effects of the Shp2 mutation, implicating Src as a primary Shp2 target during Nrg1 signaling. Our phenotypic and biochemical analyses thus demonstrate that Shp2 is an essential component in the transduction of Nrg1/ErbB signals.

Specific inhibitors of the protein tyrosine phosphatase Shp2 identified by high-throughput docking

Klaus Hellmuth, Ching Tung Lum, Martin Würtele, Marta Rosario, Walter Birchmeier, Stefanie Grosskopf (FMP), Jörg Rademann (FMP) and Jens von Kries (FMP).

The protein tyrosine phosphatase Shp2 is a positive regulator of growth factor signaling. Gain-of-function mutations in several types of leukemia define Shp2 as a bona fide oncogene. We performed a high-throughput *in silico* screen for small-molecular-weight compounds that bind the catalytic site of Shp2. We have identified the phenylhydrazonopyrazolone sulfonate PHPS1 as a potent and cell-permeable inhibitor, which is specific for Shp2 over the closely related tyrosine phosphatases Shp1 and PTP1B. PHPS1 inhibits Shp2-dependent cellular events such as hepatocyte growth factor/scatter factor (HGF/SF)-induced epithelial cell scattering and branching morphogenesis. PHPS1 also blocks Shp2-dependent downstream signaling, namely HGF/SF-induced sustained phosphorylation of the Erk1/2 MAP kinases and dephosphorylation of paxillin. Furthermore, PHPS1 efficiently inhibits activation of Erk1/2 by the leukemia-associated Shp2 mutant, Shp2-E76K, and blocks the anchorage-independent growth of a variety of human tumor cell lines. The PHPS compound class is therefore suitable for further development of therapeutics for the treatment of Shp2-dependent diseases.

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Patent

MDC 0501: Shp-2 inhibitors, pharmaceutical compositions comprising them and their use for treating phosphatase-mediated diseases. W. Birchmeier, K. Hellmuth. EP 05 090 160.2.

Daniel Besser
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Signaling Mechanisms in Embryonic Stem Cells

Embryonic stem cells (ESC) are pluripotent cells, which can proliferate indefinitely and participate in the formation of most cell types. Studies on human ESCs (hESC) provide an insight into human embryogenesis and allow the development of tools for pharmacology and regenerative medicine. The focus of our studies is the maintenance of the pluripotent state in murine ESCs (mESCs) and hESCs. We found that the Activin/Nodal pathway is activated in pluripotent hESCs and blocked upon differentiation. Pluripotent cells require these signaling regulating a specific subset of target genes. In addition, the BMP pathway is in the off-state in pluripotent cells and activated upon differentiation. This pathway counteracts the effects of Activin signaling. Moreover, it has been shown that somatic cells for various sources can be reprogrammed by transfection of four defined transcription factors, i.e. Oct4, Sox2, Klf4, and c-Myc, to a pluripotent state. These induced pluripotent stem cells (iPS) are very exciting new cell populations for stem cell research.

Role of TGFβ signaling in pluripotency of embryonic stem cells

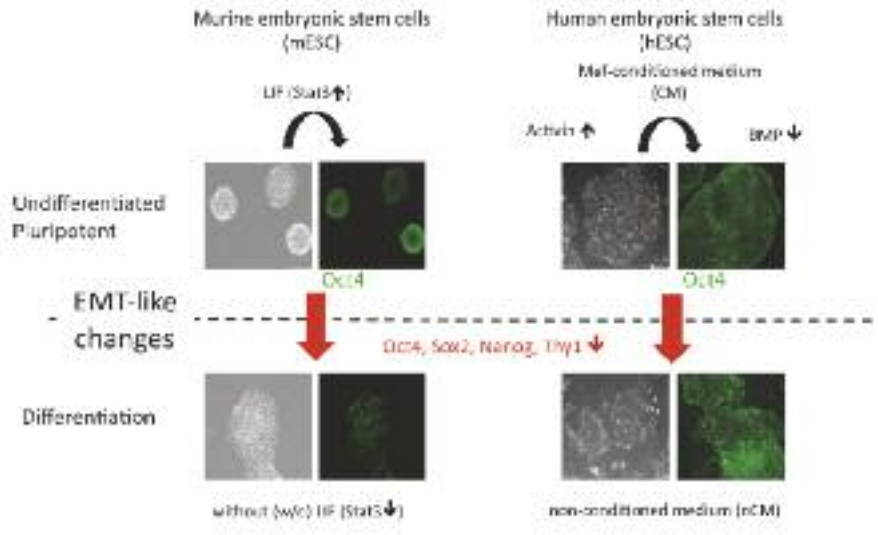
Angel Quiroga-Negreira, funded by the DFG, SPP1109 Embryonal and Tissue-specific Stem Cells and HGF-systems biology NW1 SB Cancer DKFZ.I.4

Our previous observations show that Activin/Nodal signaling is required for maintenance of the undifferentiated state in hESCs while BMP signaling induces differentiation. This raises important questions regarding the downstream target genes that are important for the regulation of pluripotency as well as differentiation. Another important question is how cross talk between these two pathways is established. In this project we have found that intrinsic Activin/Nodal and BMP signaling in hESCs controls the molecular events and the regulation of a whole set of downstream target genes. In a recent analysis of global expression profiles using Affymetrix microarrays in a dense time course in hESCs and mESCs upon differentiation, activation and inhibition of Activin/Nodal signaling and activation of BMP signaling we gained deeper insight into the regulatory events underlying these processes. Moreover, by mathematical modeling we are in the process of using this dataset for a detailed analysis of gene regulation in pluripotent ESCs and during early differentiation. We found that in hESCs, Activin A treatment under differentiating conditions leads to establishment of the pluripotency program, while inhibition of Activin signaling and BMP4 treatment strongly induces differentiation into specific cell lineages. The data also suggest that molecules that regulate cell-cell interaction and cell motility, especially molecules of the Cadherin family and molecules regulating the small GTP-binding protein Rho are regulated very early after induction of differentiation.

Regulation of Oct4: How the regulator is regulated

Sebastian Diecke and Angel Quiroga-Negreira

It is well established that three transcription factors, i.e. Oct4 (Oct3/4, Pou5F1), Nanog, and Sox2 that interact with each other, are at the core of the molecular events



The pluripotent state of murine and human embryonic stem cells (m/hESC). mESCs can be maintained undifferentiated in LIF, while hESCs require conditioned medium (CM) from mouse embryo fibroblasts (MEF) leading to activation of Activin signaling and block of BMP4 signaling. Upon differentiation, i.e. by withdrawal of LIF or cultivation in non-conditioned medium (nCM) the pluripotency transcription factor Oct4 is downregulated and we observe changes in cell morphology reminiscent to epithelial to mesenchymal transition (EMT).

maintaining the pluripotent state. However, we observe a significant decrease of Oct4 levels only several days after induction of differentiation, although molecular changes related to the differentiation can be observed during the first 24 hr. In addition, we observed in chromatin precipitation experiments that Oct4 levels binding in specific promoters decreases before a decrease in total protein level is detected. Thus, additional signaling events such as phosphorylation may have an impact on the activity of the Oct4 transcription factor complex. Interestingly, we observed a change in the charge of Oct4 protein in differentiating versus undifferentiated cells, suggesting differential phosphorylation of Oct4 upon differentiation. Comparison of Oct4 orthologe sequences from different species show a highly conserved homology surrounding the nuclear localization signal (NLS) and in the C-terminal domain containing threonine and serine residues, which are potential phosphorylation sites. We are currently testing whether phosphorylation of these sites influence the cellular distribution of Oct4 during the early differentiation and contribute to its inactivation. The regulation of Oct4 by phosphorylation may also explain why signaling pathways, such as Activin/Nodal have an impact on the regulation of pluripotency.

Deciphering mechanisms during reprogramming of mouse embryonic fibroblasts to pluripotent stem cells

Torben Redmer and Sebastian Diecke, funded by START-MSC Consortium, BMBF joint project grant

The reprogramming of murine fibroblasts by transduction of the transcription factors Oct4, Sox2, Klf4 and c-Myc has become an established method to generate pluripotent cells, but mechanisms that govern these steps are still poorly understood. We identified a population of cells that seems to be partially reprogrammed and display features of fibroblasts, although viral transcripts of the four factors are present. Using FACSorting we separated cells positive and negative for the cell surface marker SSEA-1 (stage-specific embryonic antigen-1). These two populations differ in their morphology, differentiation capacity like formation of embryoid bodies and in gene expression levels of endogenously expressed markers of pluripotent stem cells like Nanog. These findings led us conclude that expression of the four critical factors is necessary to induce reprogramming but its not sufficient for fully generation of stable iPS cells. Therefore additional mechanisms and signaling pathways play a role during this process.

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Fritzmann, J.*, Morkel, M.*, Besser, D. *, Budczies, J., Kosel, F., Brembeck, F.H., Stein, U., Fichtner, I., Schlag, P.M., and Birchmeier, W. (2009) A Colorectal Cancer Expression Profile that Includes Transforming Growth Factor β Inhibitor BAMBI Predicts Metastatic Potential. Gastroenterology, 137, 165-175 (* equal contribution).

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Genetics of Tumor Progression and Metastasis

The mammal cell cycle has been extensively studied nevertheless its intricacies remain elusive. Furthermore, the knowledge of cancer stem cells is at its beginnings. To tackle these complex questions, we modify murine ES-cells to characterize both transcriptional and signaling modules in stemness, proliferation or differentiation. Similarly, we use mouse models to define the molecular circuitry underlying cancer causing stem cells. Central to our work is the understanding of the molecular consequences of deregulation of the retinoblastoma protein (RB) and E2Fs, since in most human tumors the RB pathway is mutated leading to unfettered activity of the E2F transcription factors. To reveal how E2Fs control growth, we search for interaction partners and chromatin modifiers. We are also interested in the specific contribution of E2F3 to tumor progression by identifying novel metastasis specific targets. In summary, our goal is to understand how specific biological processes as cellular stemness, growth control and suppression of tumorigenesis are coupled.

Interaction partners and possible co-activators of E2F3

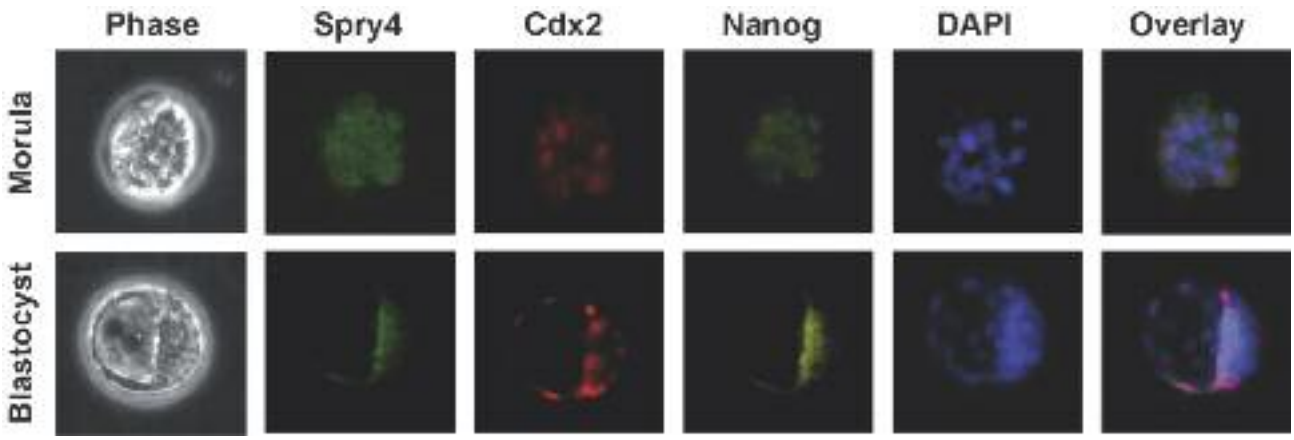
Björn von Eyss and Katharina Möllmann

The inactivation of the RB tumor suppressor pathway depends on the activity of the E2F-transcription factors, since in pRB mutant mice E2F3 contributes extensively to the ectopic S-phase and apoptosis. Depleting E2F3 alone leads to cellular growth defects but the molecular mechanism of E2F3 is not understood. Searching for specific interaction partners of E2F3, we identified a number of novel molecules, among them putative chromatin regulators. For some of these regulators we were able to show that they can be detected on E2F-regulated promoters, that they are essential for the progression through cell cycle and are co-expressed with E2F3 in human tumors. Lastly, we are using ChIP coupled to deep sequencing to reveal the whole scope of co-regulated target genes. So far, our genome wide search revealed unanticipated E2F-target genes, which will be focus of our future research.

E2F target genes in proliferation and metastasis

Kirsten Vormbrock and Sebastian Memczak

E2f3 is the only E2F amplified in human tumors, whereby high E2F3 expression marks invasiveness predicting poor patient survival. This led to the hypothesis that E2F3 regulates unique sets of target genes. To search for such targets we use a mouse strain deficient for both pRB/E2F3. In this model, merely few pRB-deficient mice develop small medullary thyroid carcinomas (MTCs), while most pRB/E2F3 deficient mutants develop metastatic MTCs. Using micro-array gene-chips we established the transcriptional profile of these MTCs. The resulting sets of transcripts are putative metastatic markers. Importantly, for four of these markers an identical pattern in human metastatic thyroid malignancies was found, a first proof that these genes are prognostic markers. Judged by chromatin immune precipitation assays, all of the genes are direct targets of E2F. Thus, we link individual E2F-action to specific E2F-target genes and the onset of metastasis.



In the morula of the early mouse embryo the Spry4 protein is expressed uniformly in all blastomeres. Upon differentiation, both Spry4 and Nanog are limited to the inner cell mass of the blastocyst. Cdx2 is a marker of the extra-embryonic lineage, showing that cells of this lineage are devoid of Spry4 or Nanog.

Proliferation and pluripotency in stem cells

Christna Chap and Gitta Blendinger

The ability to derive multiple differentiated lineages is a hallmark of embryonic stem cells (ES cells). Since ES-cell transplanted into nude mice develop primitive tumors, ES cells are viewed as an ideal *in vitro* model for early mammalian development and tumorigenesis. Using ES-cells differentiation, we identified *Sprouty4* (*Spry4*) as a novel player of the pluripotency network. Also, in ES cells *Spry4* acts as an inhibitor of the ERK pathway to regulate pluripotency. Using ChIP assays, we established that *Spry4* is transcriptionally repressed by Nanog. This repression is essential, since over-expression of Spry4 renders ES-cells insensitive to differentiation. Consistent with a role for *Spry4* in pluripotency, we find its expression restricted to the inner cell mass of mouse embryos (see Figure). Our results show that Spry4 tightly regulates several signal pathways to maintain the pluripotent state of ES cells. In the future, we will characterize specific mutants of *Spry4* that likely infer commitment to differentiation lineages. Since we believe, that Spry4 impinges upon more pathways than it is currently recognized, we intend to establish new *Spry4* mouse models allowing us to dissect cooperating signaling events in cancer and stem cells.

Determinants of cancer stem cells activity

In collaboration with Peter Wend and Walter Birchmeier

Recent evidence suggests that organ as well as cancer stem cells share core transcriptional and signaling modules. This points towards the fact that both cell types share a common ancestor. To understand if this is true, we deployed genetic mouse models and studied Wnt/β-catenin and Bmp signaling in salivary gland stem cells. Our results imply that both Bmp and proper Wnt/β-catenin signals are required to properly control cell division, apoptosis and differentiation of salivary gland stem cells. Significantly, if mutations in both pathways are joined, a tenfold increase of stem cells is observed. These stem cells are activated upon regeneration and are highly tumorigenic. In the future, we will dissect the transcriptional profiles of these stem cells to understand their specific contribution to tumorigenesis.

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Patents

MIT Case No. 8756 "ANTIBODY TO P10".

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Cell Differentiation and Tumorigenesis

Stem cells, transcription factors, & differentiation

Somatic stem cells undergo self-renewal to maintain the stem cell pool and give rise to transit amplifying progenitor cells that terminally differentiate into mature cells. Self-renewal, proliferation, and cell differentiation are interlaced genetic programs that are tightly controlled by gene regulatory proteins (transcription factors). Transcription factors downstream of signaling cascades dynamically adjust the expression of developmental genes. Some transcription factors represent nodal points in such regulatory networks of growth and differentiation. These key transcription factors may mutate and become tumorigenic by blocking differentiation or deregulating self-renewal.

We focus on the dynamic regulation of CCAAT Enhancer Binding Protein (C/EBP) α and β transcription factors and their interaction with co-factors of the epigenetic machinery and of cell division control. C/EBPs regulate genes involved in stem cell functions, proliferation, differentiation, metabolism, immunity, senescence, and tumorigenesis and determine lineage commitment in the hematopoietic system and myeloid trans-differentiation. Of particular interest is how extracellular signals instruct C/EBPs to orchestrate "stemness", cell multiplication, or lineage restricted terminal differentiation.

Lineage specific gene expression patterns are established during cell differentiation while alternative differentiation options and self-renewal are extinguished. The choreography of all these steps is essential to proper differentiation, as many malignant diseases, in particular leukemic diseases, display conflicting gene expression patterns and "multi-lineage competence", known as lineage infidelity. How "multi-lineage competence" is maintained in stem cells, how it is extinguished during lineage commitment and why it resurfaces during tumorigenic conversion are major topics of our research.

C/EBP: Structure & Function

C/EBPs comprise a family of 6 genes in vertebrates. Four members, C/EBPα,β,δ,ε, are highly related whereas two others, γ and ζ, are more divergent and display homology only in their C-terminal ~100 amino acid long basic DNA binding leucine zipper domain (bZip) that forms a bifurcated coiled-coil domain which interacts with specific cis-regulatory sites on DNA. The N-terminal part of C/EBPα,β (first ~100 amino acids) represent strong trans-activation domains (TAD) whereas the center sequences (aa ~100-200) contain regulatory domains (RD).

Knockout analysis of individual C/EBPs in mice and phylogenetic tree construction suggested that C/EBPα and C/EBPβ are the most ancient C/EBPs. Combined loss of C/EBPαβ, causes placental defects in trophoblast cells and early embryonic lethality, suggesting that they are the most important C/EBPs.

The activity of C/EBα,β is regulated by translational control and by post-translational modifications that determine combinatorial co-factor interactions with the gene regulatory and the epigenetic machineries. Multiplicity of signaling regulated modifications permit many combinatorial interactions and plasticity of developmental processes to be accomplished with a limited set of regulators.

Translational regulation of C/EBPs

Valérie Bégay, Jeske J. Smink, Klaus Wethmar

C/EBPα and C/EBPβ transcripts harbor small upstream open reading frames (uORF) in their mRNAs that sense the activity of translation initiation factors and relay initiation to alternative in-frame start sites. As a result, truncated proteins that lack part of the N-terminal sequences are generated. Besides C/EBPs, many transcripts of key regulatory genes involved in growth, differentiation, and proliferation harbor uORFs, suggesting

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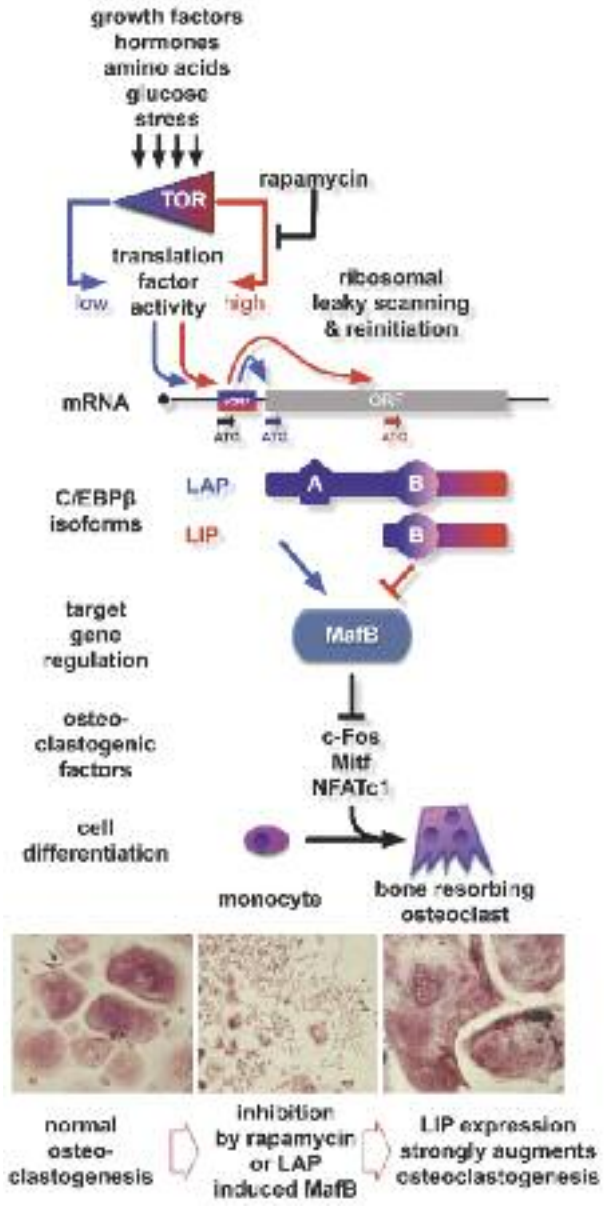
an important role of uORF-mediated translational control in mammalian development and physiology. Nevertheless, genetic models in mammals are lacking to explore the physiological relevance of uORF-regulated translation initiation.

Truncated C/EBP isoforms sustain proliferation, whereas full-length forms are inhibitors of cell division. Previously, we showed that anaplastic large cell lymphoma and Hodgkin Lymphoma express predominantly truncated C/EBPβ. Rapamycin, an antibiotic that inhibits mTOR signaling, shuts down the truncated C/EBP isoform and concomitantly inhibits growth in both types of lymphomas. Ectopic expression of truncated C/EBPβ restored proliferation, suggesting that truncated C/EBPβ represents a translationally controlled oncogene.

By targeted recombination we have now generated mouse C/EBPβ mutants that express distinct C/EBPβ isoforms. The murine mutants revealed that C/EBPβ isoform switching is important during liver regeneration, bone homeostasis and tumorigenesis. Mice deficient for the C/EBPβ uORF initiation codon (C/EBPβΔuORF) fail to initiate translation of the auto-antagonistic, truncated "LIP" C/EBPβ isoform. Livers of C/EBPβΔuORF mice displayed defective regeneration. After partial hepatectomy, delayed and blunted entry of hepatocytes into S-phase, persistent repression of E2F-regulated genes, and hyperactivation of acute phase response genes became evident. Thus, switching to the truncated isoform is important during liver regeneration and in shutting off acute phase response.

C/EBPβ deficient mice and mice expressing only the truncated "LIP" isoform (LIP knock-in mice, L/L) both display increased bone resorption. This is due to enhanced differentiation of the bone resorbing cell, the osteoclast. Failure to switch back to the long C/EBPβ isoform augmented osteoclastogenesis. Indeed, ectopic expression of the long isoform "LAP" in monocytes inhibited formation of multi-nucleated osteoclasts. Rapamycin, an inhibitor of mTOR signaling that increases the "LAP" over "LIP" ratio, also inhibited osteoclastogenesis in wild



Model of integrated control of osteoclastogenesis by C/EBPβ.

Top to Bottom: *Long (LAP) and truncated (LIP) C/EBPβ isoforms are generated from a single mRNA. The ratio between LAP and LIP is determined by the activity of translation initiation factors, which are downstream of growth factor, hormone, nutrient, and stress signaling pathways. The mammalian Target Of Rapamycin (mTOR) kinase integrates these signals and plays an important role in adjusting the LAP/LIP ratio (blue: low activity; red: high activity). The LAP isoform induces MafB, an inhibitor of osteoclastogenesis that suppresses the activity of several osteoclastic regulators (Mitf, Fos, NFATc1). Osteoclast precursors that lack C/EBP or that express LIP only, display strongly augmented formation of giant osteoclasts that readily destroy bone substance.*

type but not in C/EBP deficient or L/L osteoclasts that both can not switch to "LAP" expression. Profiling analysis of the transcriptome showed that rapamycin treatment or ectopic expression of "LAP" activated expression of MafB, a negative regulator of osteoclastogenesis. This suggested that restriction of osteoclastogenesis by "LAP" or rapamycin is dependent on MafB. In accordance, knock-down of MafB induced osteoclastogenesis, regardless of rapamycin treatment or C/EBP isoform expression. Altogether, the data showed that differential regulation of MafB gene expression by C/EBPβ isoforms determines the balance of bone turnover and that the control of C/EBPβ isoform translation represents a target for osteoporosis treatment.

Post-translational C/EBP modifications and epigenetic functions

Elisabeth Kowenz-Leutz and Ole Pless

Several years ago, we found that C/EBPα,β may instruct even non-hematopoietic cells, such as skin fibroblasts, to express myeloid genes. Others have shown that C/EBPs may reprogram lymphocytes into myeloid cells. Accordingly, C/EBPs entail epigenetic competence and gene regulatory functions to determine cell fate. We had also shown that cellular signaling cascades regulate the activity of C/EBPβ to convert extracellular information into gene regulation. C/EBPβ is a ras/MAPkinase signal sensitive transcription factor that regulates genes involved in metabolism, proliferation, differentiation, immunity, senescence, and tumorigenesis. The functional capacity of C/EBPβ is governed by protein interactions that depend on post-translational C/EBPβ modifications. In a proteome-wide interaction screen, the histone-lysine N-methyltransferase, H3 lysine-9 specific 3 (G9a) was found to directly interact with the C/EBPβ transactivation domain (TAD). G9a, but not a defective G9a mutant abrogated the transactivation potential of wild type C/EBPβ. Metabolic labeling showed that C/EBPβ is post-translationally modified by methylation of its TAD. A conserved lysine residue (K39) in the C/EBPβ-TAD served as a substrate for G9a mediated methylation. A C/EBPβ K39 alanine exchange mutant was resistant to G9a mediated inhibition and conferred super-activation of myeloid genes. These data identified C/EBPβ as a direct substrate of G9a that alters the functional properties of C/EBPβ by post-translational lysine methylation.

Mass spectrometry of cell derived C/EBPβ (in collaboration with Gunnar Dittmar, MDC) revealed extensive methylation of N-terminal arginine residues in C/EBPβ. The protein arginine methyl-transferase 4 (PRMT4) was found to interact with C/EBPβ and to di-methylate the conserved arginine residue (R3) in the C/EBPβ TAD. Phosphorylation of the regulatory domain of C/EBPβ by ras/MAPkinase signaling abrogated the interaction between C/EBPβ and PRMT4. Differential proteomic screening with R3-methylated and un-methylated C/EBPβ peptides, protein interaction studies, and mutational analysis revealed that methylation of R3 constrained the interaction between C/EBPβ with SWI/SNF and Mediator complexes. Both complexes play essential roles in chromatin remodeling and transcription initiation by polymerase II and were previously implicated in C/EBPβ functions. Mutation of the R3-C/EBPβ methylation site alters the ability of C/EBPβ to induce myeloid and adipogenic differentiation. Thus, phosphorylation of the transcription factor C/EBPβ couples ras signaling to arginine methylation and regulates the interaction of C/EBPβ with epigenetic gene regulatory protein complexes during cell differentiation.

A number of implications and conceptual advances are contained in these results. An "indexing code" of post-translational transcription factor modifications has recently been suggested (Sims & Reinberg, 2008), although experimental evidence is currently still scarce. Covalent modifications by "writers" (here, R-methylation by PRMT4, and K-methylation by G9a) determine modification dependent docking of "readers" (SWI/SNF; Mediator; G9a). Our results imply that writing/reading such an indexing code is downstream of receptor tyrosine kinase signaling, relaying signals to epigenetic events that finally determine cell fate. This concept is an important extension of the Histone Code hypothesis to non-histone transcription factor proteins, which come first in gene regulation and before chromatin modifications occur. Many mechanistic (gene regulation in development and disease) and medical issues (pharmacology) are implied.

Functional interactions between C/EBPalpha and E2F-DP complexes

Katrin Zaragoza and Qingbin Liu

C/EBPα coordinates proliferation arrest and differentiation in many cell types. C/EBPα transactivates lineage specific differentiation genes and inhibits proliferation by repressing E2F-regulated genes. The myeloproliferative C/EBPαBRM2 mutant serves as a paradigm for recurrent human C-terminal bZip C/EBPα mutations that are involved in acute myeloid leukemogenesis. BRM2 fails to repress E2F and fails to induce adipogen-

esis and granulopoiesis. We showed that C/EBPα or BRM2 interact with the dimerization partner (DP) of E2F and that BRM2 displays enhanced interaction with the E2F-DP complex. Augmented interaction with E2F-DP prevents binding of C/EBP to its cognate sites on DNA and thus prevents transactivation of C/EBP target genes. Repression of C/EBPα by E2F-DP occurs independently of pocket proteins, including the retinoblastoma protein Rb and the related p107,and p130. Although BRM2 is more susceptible to E2F-DP repression it retains transactivation potential and differentiation competence, as disclosed by knock-down of E2F or DP expression. These data suggested that a tripartide balance between C/EBPα, E2F/DP, and pocket proteins control proliferation and differentiation and that E2F-DP mediated block of C/EBPα functions may play a role in tumorigenesis.

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Cancer, Stem Cells, and Transcription Factors

Stem cells are capable of life-long self-renewal and multi-lineage differentiation. The formation of early progenitors of the myeloid lineage from stem cells is orchestrated by a relatively small number of transcription factors. Among them are PU.1, CCAAT/enhancer binding protein α (C/EBPα), growth factor independent 1 (GFI1), interferon-regulatory factor 8 (IRF8), Runt-related transcription factor 1 (RUNX1) and stem-cell leukemia factor (SCL). Mice in which these genes have been knocked out displayed profound hematopoietic defects. Moreover, these transcription factors were shown to regulate a broad range of pivotal target genes, thereby directly programming precursors to differentiate along a complex developmental pathway. A block in normal differentiation is a major contributing factor towards the development of solid tumors and leukemias and cells from leukemia patients frequently harbor mutated or dysregulated transcription factor genes. This suggests that altered transcription factor activity is a major driving force behind the pathology of transformation and the development of cancer stem cells.

Dynamic PU.1 expression in hematopoiesis and leukemia

One of the main interests of our laboratory is to understand how transcription factors direct normal stem cell functions, such as self-renewal and differentiation, how they program precursors to adopt a certain lineage choice and how disruption of transcription factor activity leads to cancer (stem) cell transformation. Using both transgenic and knockout mouse models, we are particularly interested in discovering crucial molecular up- and downstream mechanisms that regulate the expression and function of transcription factors. A current research focus in our laboratory is on PU.1. The Ets-family member PU.1 is essential for both myeloid and lymphoid lineages. PU.1 knockout mice exhibit early lethality and lack of B-lymphocytes and mature myeloid cells in fetal livers. In addition, PU.1 is important for HSC self-renewal and differentiation into the earliest myeloid and lymphoid progenitors. Furthermore, PU.1 must be properly downregulated in early thymocytes to allow normal T cell development. It was shown that graded changes in PU.1 concentrations have drastic effects on lineage fate decisions. Therefore, a greater understanding of PU.1 gene regulation is the key to deciphering its role in normal hematopoiesis and malignant transformation.

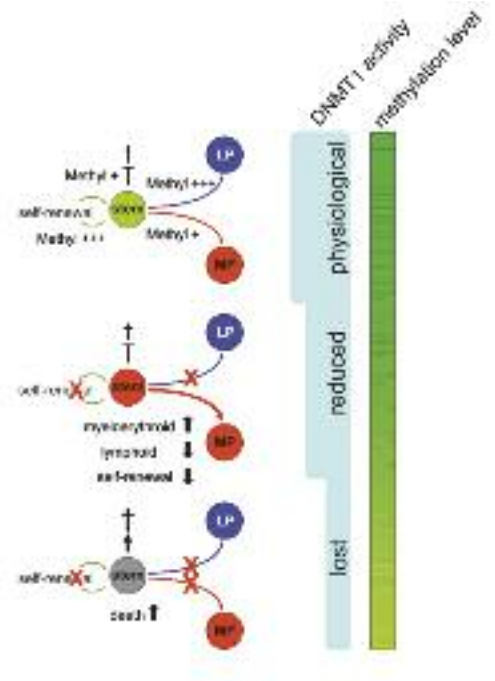
We identified a novel distal DNA element (termed upstream regulatory element, URE) which is pivotal for proper PU.1 gene expression *in vivo*. We generated URE deficient mice ($URE^{\Delta/\Delta}$) using targeted recombination in ES cells. Remarkably, URE deletion led to a marked decrease in PU.1 expression in HSCs, macrophages and B cells, but an increase in PU.1 expression in early thymocytes. This demonstrated that the URE has an essential cell context specific regulator function, and directs PU.1 expression as an enhancer in myeloid and B-lymphoid cells but functions as a repressor in T cells. Due to these profound effects of URE deletion on PU.1 expression,

URE$^{\Delta/\Delta}$ mice regularly developed aggressive hematopoietic malignancies, such as acute myeloid leukemia, T cell lymphoma and B1 cell chronic lymphoid leukemia. Results from the URE$^{\Delta/\Delta}$ animal model provided the first demonstration that interference with the fine-tuned regulation of a single transcription factor, through disruption of a key *cis*-regulatory element, can be sufficient to initiate the formation of cancer stem cells and subsequent tumor development.

Epigenetic control of hematopoietic stem cell function

Methylation of CpG dinucleotides within the DNA is a major epigenetic modification, which in mammals is controlled by at least 3 different DNA-methyltranferases (DNMTs): DNMT3a and -b for *de novo* methylation and DNMT1 for methylation maintenance. The impact of methylation on stem cell features has been studied in embryonic stem (ES) cells, but little is known about its function in somatic stem cells. Recent advances in the genome-wide mapping of DNA methylation demonstrated that methylation of CpGs are dynamic epigenetic marks that undergo extensive changes during cellular differentiation. However, whether and how these changes are required for cell fate choice, in particular for that of stem cells, remained unknown. Moreover, altered methylation is a hallmark of cancer, and drugs targeting methylating enzymes are used in cancer therapy. The relationship between tumor-associated alterations in methylation and cancer stem cell properties was still elusive.

We could show that alternative functional programs of HSCs are governed by gradual differences in the methylation level (Bröske et al. in press). Constitutive methylation is essential for HSC self-renewal, but dispensable for homing, cell cycle control and suppression of apoptosis. Remarkably, HSCs from mice with reduced DNMT 1 activity fail to suppress key myeloerythroid regulators and as a consequence can differentiate into myeloerythroid but not into lymphoid progeny. We revealed that a similar methylation dosage effect controls stem cell function in leukemia. Thus, our data identified DNA methylation as an essential epigenetic mechanism to protect stem cells from premature activation of predominant differentiation programs and suggest that methylation dynamics determines stem cell functions in tissue homeostasis and cancer (Figure 1). Consequently, these results provide the hope that demethylating drugs may be instrumental to impair the function of cancer stem cells in cancer therapy.



Model of DNA methylation dosage effects on stem cell multipotency. In the presence of physiological DNMT1 concentrations (top panel), stem cells are competent of a diverse functional repertoire which includes self-renew, differentiation into myeloid or lymphoid progeny (MP, LP) and suppression of apoptosis. The molecular basis of this diversity lies in a tightly balanced expression of genes that control self-renewal with genes that prime for certain developmental fates. Reduction in DNMT1 levels (middle panel) narrows the functional options of stem cells by lifting the suppression of a predominant myeloerythroid gene program via insufficient promoter methylation. As a consequence, expression of self-renewal and lymphoid genes becomes blocked. Finally, complete loss of DNMT1 (bottom panel) blocks the entire functional stem cell repertoire and causes the rapid elimination of the stem cell pool by cell-autonomous induction of apoptosis. Methyl +++: high methylation level required, Methyl +: low methylation level required.

Selected publications

Steidl U, Rosenbauer F, Verhaak RGW, Gu X, Out HH, Bruns I, Steidl C, Costa DB, Klippel S, Wagner K, Aivado M, Kobbe G, Valk PJ, Passegué E, Libermann TA, Delwel R, and Tenen DG. (2006) Essential role of Jun family transcription factors in PU.1-induced leukemic stem cells. Nature Genet 38, 1269-1277.

Scheller M, Huelsken J, Rosenbauer F, Taketo MM, Birchmeier W, Tenen DG, and Leutz A. (2006) Hematopoietic stem cell and multi lineage defects by β-catenin activation. Nature Immunol 7, 1037-1047.

Rosenbauer F, Owens BM, Yu L, Tumang JR, Steidl U, Kutok JL, Clayton LK, Wagner K, Scheller M, Iwasaki H, Liu C, Hackanson B, Akashi K, Leutz A, Rothstein TL, Plass C, and Tenen DG. (2006) Lymphoid cell growth and transformation are suppressed by a key regulatory element of the gene encoding PU.1. Nature Genet 38, 27-37.

Rosenbauer F and Tenen DG. (2007) Transcription factors in myeloid development: Balancing differentiation with transformation. Nature Rev Immunol 7,105-117.

Bröske AM, Vockentanz L, Kharazi S, Huska M, Mancini E, Scheller M, Enns A, Prinz M, Jaenisch R, Nerlov C, Leutz A, Andrade-Navarro MA, Jacobsen SEW, Rosenbauer F. (2009). DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. Nature Genetics in press.

Peter M. Schlag

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Surgical Oncology

Our group has been focusing on the establishment of predictive and prognostic gene signatures and expression profiles for analysis of metastases and drug resistance in colorectal cancer. The data provide the basis for novel therapeutic concepts in tumor treatment that are applicable and validated in the clinic. We were successful in identifying new metastases-associated genes, their importance for cancer metastases and their function in signaling pathways. We isolated and characterized the new gene MACC1, revealed S100A4/metastasin as novel target gene of the β-catenin pathway and analyzed the interplay of BMP-4 and Bambi in the context of β-catenin signaling. This provided insights into the tight association between early and late events of the metastasis process particularly in colorectal cancer, and opens opportunities for targeted therapies. In the identification of patient-individualized gene expression profiles for improved diagnosis and prediction regarding metastases formation and patient survival, the achievements were made in close collaboration with the groups of W. Birchmeier and M. Lipp.

In parallel to our achievements in understanding of new key regulators and molecular mechanisms in metastasis, we significantly advanced in the clinical development of local nonviral gene transfer for effective and applicable cancer gene therapy. We successfully performed a clinical "proof of principle“ phase I gene transfer trial at the ECRC using jet-injection for transfer of naked DNA. In conclusion of this trial we made great efforts to further improve safety and efficiency of nonviral vector systems and tested their therapeutic potential for clinical application.

Classification of gastrointestinal carcinomas by gene expression profiling

W. Kemmner, W. Qing, S. Förster, Q. Wang, P. M Schlag. In cooperation with M. Yashiro (Department of Surgical Oncology, Osaka City University Graduate School of Medicine,Osaka, Japan), M. Vieth (Institute of Pathology, Klinikum Bayreuth, 95445 Bayreuth), P. Malfertheiner (Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University Magdeburg, 39120 Magdeburg)

The aim of this project was to find gene signatures that allow a more precise differentiation of prognostic subtypes of gastrointestinal carcinomas. RNA was extracted from 60 colorectal, 72 gastric and 130 esophageal cancers and precancerous lesions respectively (e.g. GERD). After quality controls of the microarray data, ensuring the comparability of the different arrays, normalization and data analysis was performed. Microarray results were validated by qRT-PCR analysis (TaqMan) and by immunohistochemistry.

In each of the three cancer entities differentially expressed genes were found which allow e.g. to discriminate between carcinoma subtypes. In the case of esophageal neoplasias we were able to describe a new potentially "high risk" group of Barrett's esophagus, in gastric carcinomas we described new candidate genes with prognostic relevance and in colorectal cancer tissue we identified a set of 42 genes which are significantly upregulated in patients who are highly at risk for recurrence after radical surgery. Most of the identified

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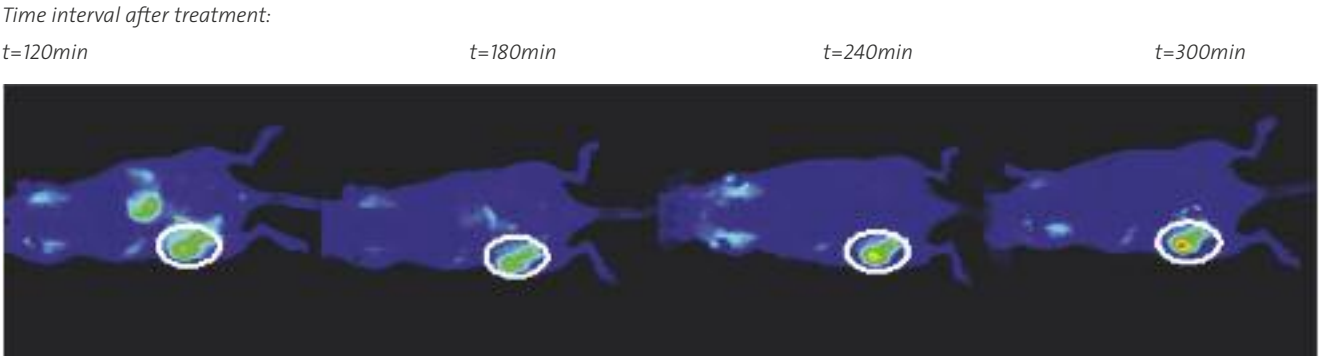


PpIX-fluorescence in tumor xenografts. *Human breast carcinoma cells MDA-MB-435 were injected subcutaneously (s.c.) into the fat pad of nude mice. Treatment of xenografts with liposomes, ALA or FECH siRNA alone showed little nonspecific fluorescence. Dramatic enhancement of PpIX fluorescent signals from the areas of xenografted tumors were detected in mice which were treated with folate-PEG cationic lipoplexes containing FECH-1140 siRNA followed by a single dose of 15mg/kg ALA. Localized fluorescent hotspots were detected at the sites of the millimeter-sized tumors. Circles indicate the xenografted tumors*

genes are known to have a tumor biologic function and/or are related to pathways.

Silencing of human ferrochelatase (FECH) leads to abundant accumulation of fluorescent protoporphyrin-IX (PpIX) in colorectal carcinoma cells - a new molecular amplification approach for early imaging of tumors

W. Kemmner, K. Wan, P. M. Schlag. In cooperation with Bernd Ebert (Department of Biomedical Optics, Physikalisch-Technische Bundesanstalt Berlin) and R. Haag (Institute for Chemistry and Biochemistry, Free University Berlin)

Silencing of FECH leads to an endogenous fluorescence by affecting the cellular heme metabolism. Application of siRNA may provide a general means for cellular imaging. Any nucleated human cell requires heme for heme-containing enzymes essential for the cellular energy metabolism. The last step of heme synthesis is incorporation of iron into Protoporphyrin IX (PpIX) that takes place in the mitochondria catalyzed by the enzyme FECH. PpIX is a fluorescing and photodynamically active chromophore which can be used for detection and treatment of neoplasias. Quantitative RT-PCR of human tissue samples revealed a significant down-regulation of FECH expression in various gastrointestinal carcinomas. Experimentally induced knock down of FECH expression in carcinoma cells by RNA-interference (siRNA) led to an accumulation of fluorescent PpIX of more than 50-fold. Moreover, PpIX fluorescence was dramatically enhanced in xenografted tumors of nude mice treated with lipoplexes containing FECH-siRNA or with siRNA bound to Polyglycerol cationic polymers. FECH-siRNA silencing might be a versatile tool for molecular imaging and cancer treatment. We reported for the first time the functional use of siRNA for cellular imaging. The presented approach exhibits no relevant toxicity, because siRNA-silencing of FECH led to an endogenous and non-toxic fluorescence by affecting the cellular heme metabolism.

MACC1, a newly identified gene regulates HGF/Met signaling and predicts colon cancer metastasis

U. Stein, W. Walther, F. Arlt, J. Smith, P.M. Schlag. In cooperation with W. Birchmeier, I. Fichtner

We identified the novel gene MACC1 (Metastasis-Associated in Colon Cancer 1) in subjects with colon cancer by genome-wide expression analysis in primary and metastatic carcinomas, adenomas and normal tissues. MACC1 is located on chromosome 7. It encodes a protein of 852 amino acids with domains that enable MACC1 for

protein-protein interactions. We found several sites for serine/threonine and tyrosine phosphorylation; features that predestine MACC1 as a signal transducer.

We identified the gene Met as transcriptional target of MACC1. Following application the hepatocyte growth factor (HGF), MACC1 translocates from the cytoplasm into the nucleus, binds to the Met promoter, and induces its transcription. The HGF/Met pathway is particularly involved in colon cancer metastasis. Thus, we identified MACC1 as a major regulator of this important cellular metastasis pathway.

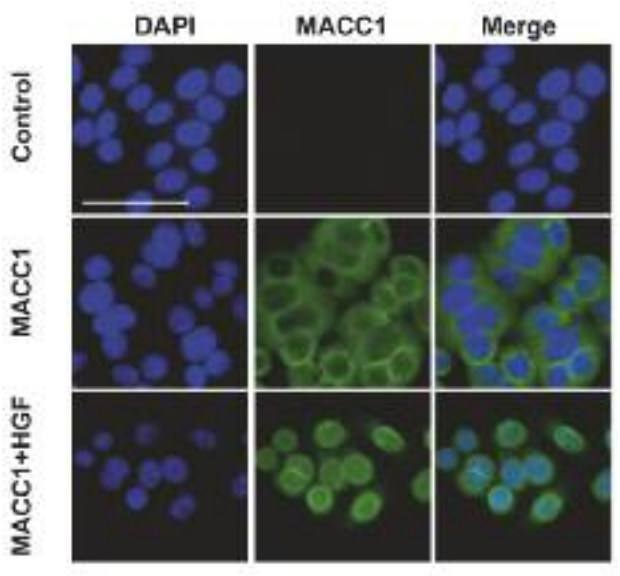
MACC1 induces migration, invasion, proliferation, colony formation, and HGF-mediated scattering in cell culture. These functional MACC1 effects were reverted by MACC1- or Met-specific siRNA. Cells with mutant MACC1 protein interaction domains did neither show MACC1-induced cell motility nor proliferation. Subcutaneous, orthotopic or intrasplenic transplantation of MACC1-expressing colon cancer cells in mice resulted in elevated tumor growth and increased numbers of distant metastases. MACC1- or Met-specific shRNA led to lowered numbers of metastases in vivo.

MACC1 was overexpressed in human colon cancer compared to normal tissues. Importantly, MACC1 expressions were significantly higher in those tumors, that metachronously metastasized compared to those, which did not metastasize within up to 10 years. MACC1 levels predict the probability of metastasis linked to metastasis-free survival. The correct positive or negative prediction for metastasis was 74% and 80%, respectively. The 5-year-survival was 80% for patients with low MACC1, compared to 15% for patients with high MACC1 expressions. MACC1 in tumor specimens is an independent prognostic indicator for metastasis and metastasis-free survival. For clinical practice, MACC1 is an important prognostic gene for early identification of high-risk subjects with colorectal cancer, and a promising new target for intervention of metastasis formation.

Inhibition of colon cancer metastasis by small molecules targeting the metastasis progressor S100A4

U. Stein, U. Sack, W. Walther, P.M. Schlag. In cooperation with W. Birchmeier, I. Fichtner (MDC), N. Scudiero, M. Selby (SAIC, Frederick, MD), Robert H. Shoemaker (National Cancer Institute-Frederick, MD)

The calcium-binding protein S100A4 is involved in metastasis formation and aggressive tumor growth in many types of cancer. We identified S100A4 as a Wnt/β-catenin target gene which induces migration, invasion and angiogenesis. Hence, its suppression bears potential for therapeutic intervention of metastasis.



HGF-induced translocation of MACC1 from the cytoplasm into the nucleus in human colon cancer cells, detected by immunofluorescence (bar 50 μm). 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 15:59-67, 2009

Here we report the identification of small molecule inhibitors which significantly reduce S100A4 expression in colorectal carcinoma cells. These inhibitors were identified from a high throughput screening of 1,280 compounds, employing the S100A4 gene promoter reporter system, and by targeting key molecules of the Wnt/β-catenin signaling pathway.

Functional assays with selected and validated compounds revealed that proliferation rates were diminished upon treatment while cell viability was only slightly affected. More strikingly, migration and invasion rates of treated cells were significantly decreased by these inhibitors, but could be rescued by overexpressing S100A4 cDNA. The impact of these small molecules on metastasis formation in vivo was demonstrated with smaller and fewer metastases per animal. In summary, our findings present a new strategy to restrict S100A4 induced metastasis formation in colon cancer.

Clinical application of nonviral gene therapy for local treatment of solid tumors

W. Walther, D. Kobelt, R. Siegel, J. Aumann, S. Burock, U. Stein, P. M. Schlag. In cooperation with M. Dietel (Institute of Pathology, Charité), M. Schleef (PlasmidFactory, Bielefeld), A. Menne (EMS Medical, Nyon, Switzerland)

For the delivery of naked DNA into cells or tissues a great variety of procedures is employed in vitro and in vivo. Among other physical delivery systems jet-injection has developed to an applicable gene transfer technology, which allows gene transfer of small amounts of naked DNA into different tissue types with deeper penetration and improved dispersion.

At the Clinic for Surgical Oncology, Charité, Berlin a phase I clinical trial (DeReGe 62) was conducted to evaluate safety, feasibility and efficiency of intratumoral jet-injection gene transfer of β-galactosidase (LacZ)-reporter expressing plasmid-DNA into skin metastases from breast cancer and melanoma. Seventeen patients were enrolled and treated with jet-injection into a single cutaneous lesion. In the study the safety and efficiency of the jet-injection gene transfer was shown. The study revealed efficient LacZ mRNA- and protein-expression in all treated lesions and rapid clearance of plasmid-DNA from patient's blood. The treatment was well tolerated by all patients and no side effects were experienced, indicating clinical applicability of this nonviral approach for local tumor gene therapy.

Use of the minimalistic MIDGE vector for improved safety, gene transfer and expression in clinical application of gene therapy

W. Walther, S. Burock, D. Kobelt, J. Aumann, U. Stein, P. M. Schlag. In cooperation with B. Wittig and M. Schmidt (MOLOGEN AG, Berlin), U. Trefzer (Charité) and I. Fichtner

In result of our clinical gene transfer trial we currently make great efforts for further optimization of the nonviral vector by removing unnecessary sequences for the improvement of transfer- and expression efficiency. The minimalistic immunologically defined gene expression (MIDGE, Mologen AG, Berlin) vector system provides a nonviral linear DNA molecule that is depleted of any bacterial origin, antibiotic resistance sequences or replication backbone sequences. This makes MIDGE of high value with respect to regulatory requirements for product safety in clinical applications. Thus, MIDGE vectors are substantially reduced in size, resulting in higher transfer efficiencies and improved transgene expression. Our in vitro analyses in different human tumor cell lines revealed pronounced increase in transfer efficiency and in transgene expression of the MIDGE vector system compared to plasmid-based vectors. The use of this system for expression of therapeutic genes, such as human TNF-α, mediated high-level expression in vitro and more importantly in vivo and resulted in effective antitumoral effects. These expression characteristics make MIDGE a good candidate for use in clinical gene therapy applications.

Based on our pre-clinical and clinical experiences on nonviral gene transfer a new phase I trial will be initiated to evaluate safety and efficiency of nonviral jet-injection application of the TNF-α expressing MIDGE-based vector in patients with skin metastasis from melanoma. This dose-escalation study will reveal, whether the MIDGE-vector is efficiently expressing TNF-α after the intratumoral application by jet-injection. It will be important to correlate the applied vector dose and the amount of TNF-α expressed in the tumor lesion. The safety of the expressed TNF-α and the vector-DNA dose applied will be examined and will be tested in a clinical phase II trial, to evaluate the safety and efficacy of this approach for the combined modality treatment of patients with malignant melanoma.

Selected Publications

Stein, U., Walther, W., Arlt, F., Schwabe, H., Smith, J., Fichtner, I., Birchmeier, W., Schlag, P.M. (2009) MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. Nat Med. 15, 59-67

Fritzmann J, Morkel M, Besser D, Budczies J, Kosel F, Brembeck FH, Stein U, Fichtner I, Schlag PM, Birchmeier W. (2009) A colorectal cancer expression profile that includes transforming growth factor beta inhibitor BAMBI predicts metastatic potential. Gastroenterology 137; 165-175

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Stein, U., Arlt, F., Walther, W, Smith, J., Waldman, T., Harris, E.D., Mertins, S.D., Heizmann, C.W., Allard, D., Birchmeier, W., Schlag, P.M., and Shoemaker, R.H (2006). The metastasis-associated gene S100A4 is a novel target of β-catenin/T-cell factor signalling in colon cancer. Gastroenterology 131, 1486-1500.

Patents

Kemmner W, Schlag PM
Microarray für das Expression Profiling der kompletten zellulären Glykosylierungsmaschinerie
Patent application: 9.10.2008

Stein U, Lange C, Walther W, Schlag PM.
Use of the human LRP/MVP promoter for a vector that can be induced by therapy.

Patent	EP 1332219	10.5.2006
	US 7,297,535	20.11.2007

Stein U, Schwabe H, Walther W, Schlag PM.
Verwendung des neu-identifizierten Gens 7a5/Prognostin für Tumordiagnostik und Tumortherapie.
7a5/Prognostin and its use for tumor diagnostics and therapy.
Patent application US 10/564,823 2006

Stein U, Walther W, Arlt F, Schlag PM.
Methods for diagnosing metastasis by analysing mutations in β-catenin.

Patent application	US 60/847,547	2006
Patent application	EP 008333	2007

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Cancer Genetics and Cellular Stress Responses in Pathogenesis and Treatment of Lymphatic Malignancies

Our research program is driven by our interest in cellular stress responses (so called 'failsafe mechanisms') that may serve as anti-tumor barriers when challenged by transforming oncogenes, and, in turn, must be bypassed or inactivated before a full-blown malignancy can actually form. Importantly, ultimate stress responses such as apoptosis or cellular senescence – both terminal 'cell-cycle exit' programs – do not only counter tumorigenesis, but are utilized as chemotherapy-induced stress responses as well. Hence, principles of oncogenesis and mechanisms of treatment sensitivity seem to critically overlap and impinge on each other during tumor formation, cancer therapy and relapsed or progressive disease conditions. Moreover, recent evidence points towards interferences between cell-autonomous failsafe programs and the tumor environment, which, in turn, evoke feedback mechanisms that may significantly alter tumor biology. To test the impact of genetic 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 lesions.

FoxO transcription factors suppress Myc-driven lymphomagenesis via direct activation of ARF

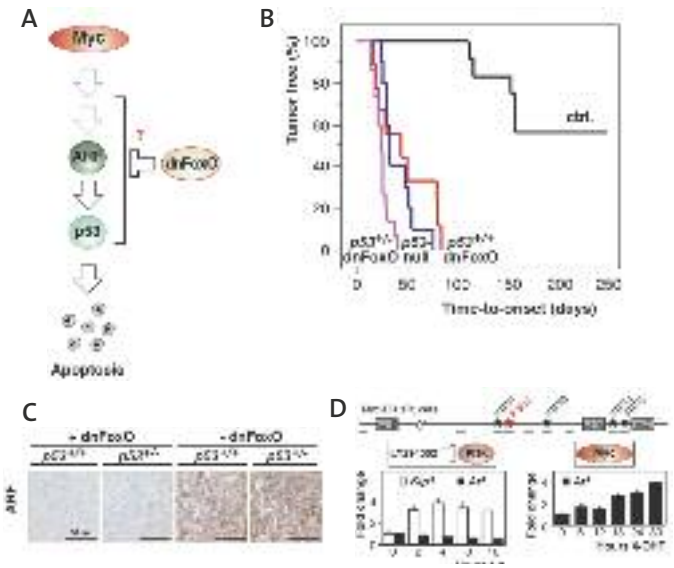
Soyoung Lee and collaboration partners

FoxO transcription factors regulate numerous cellular functions including proliferation, stress sensitivity, and cellular survival. Conditional co-deletion of FoxO familiy members 1, 3 and 4 in the absence of a defined oncogenic stimulus unveiled a context-dependent cancer-prone phenotype after long latencies. We investigated in an oncogene-driven setting, namely the Eμ-*myc* transgenic mouse model, whether ablation of FoxO function via transduction of hematopoietic stem cells with a dominant-negative FoxO moiety would give rise to accelerated tumor onset. We not only demonstrated a tumor-suppressive role of FoxO transcription factors in Myc-induced lymphomagenesis but showed genetically an interference of FoxO function with the p53 pathway. Precisely, we found FoxOs to bind to the INK4a/ARF locus and to induce ARF expression when Myc is constitutively expressed. Thus, FoxO transcription factors have tumor-suppressive potential and act by promoting ARF expression in response to oncogenic activation – a pivotal pathway with FoxOs now identified to mediate the missing link between Myc activation and ARF induction.

Oncogenic signaling evokes a DNA damage response that is selected against in manifest lymphomas

Maurice Reimann, Ines Schildhauer, Bianca Teichmann, Bernd Dörken and collaboration partners

In addition to the *ARF/p53* pathway, the DNA damage response (DDR) has been recognized as another oncogene-provoked anti-cancer barrier in early human tumorigenesis leading to apoptosis or cellular senescence. DDR mutations may promote tumor formation,



Myc and FoxO transcription factors co-operate in tumor-suppressive ARF induction. *We tested the questions whether FoxO transcription factors possess tumor-suppressive potential in Myc-driven tumor development and whether a dominant-negative FoxO moiety (dnFoxO) may act via interference with the ARF/p53 pathway (**A**). Eμ-myc transgenic, p53$^{+/+}$ or p53$^{+/-}$ hematopoietic stem cells were stably transduced with dnFoxO or mock, and lymphoma onset was monitored. Note that p53$^{+/+}$,dnFoxO lymphomas formed much faster than controls (i.e. p53$^{+/+}$ lymphomas without dnFoxO), and arose virtually indistinguishable from the p53$^{+/-}$ group were lymphomas typically lose the remaining p53 allele ('p53null') (**B**). ARF expression is detectable in lymphoma sections independent of their p53 status, but only in the absence of the dnFoxO moiety (**C**). FoxO transcription factors bind to the FBS2 consensus site in the INK4a/ARF promoter, but only induction of Myc, not activation of FoxOs by the PI3 kinase inhibitor LY294002 (both resulting in equal binding of FoxOs to FBS2 [data not shown]), promote ARF expression (**D**).*

but their impact on treatment outcome remains unclear. In this study, we generated Atm (ataxia telangiectasia mutated)-proficient and -deficient B-cell lymphomas in *Eμ-myc* transgenic mice to examine the role of DDR defects in lymphomagenesis and treatment sensitivity. *Atm* inactivation accelerated development of lymphomas, and their DNA damage checkpoint defects were virtually indistinguishable from those observed in *Atm*$^{+/+}$-derived lymphomas that spontaneously inactivated the pro-apoptotic Atm/p53 cascade in response to Myc-evoked reactive oxygen species (ROS). Importantly, acquisition of DDR defects, but not selection against the ARF pathway, could be prevented by lifelong exposure to the ROS scavenger N-acetyl-cysteine (NAC) *in vivo*. Following anticancer therapy, DDR-compromised lymphomas displayed apoptotic, but, surprisingly, no senescence defects, and achieved a much poorer long-term outcome when compared to DDR-competent lymphomas treated *in vivo*. Hence, Atm eliminates pre-neoplastic lesions by converting oncogenic signaling into apoptosis, and selection against an Atm-dependent response promotes formation of lymphomas with predetermined treatment insensitivity.

Apoptotic lymphoma cells trigger TGF-β secretion of host macrophages that results in tumor-suppressive lymphoma cell senescence

Maurice Reimann, Soyoung Lee, Jan Dörr, Bernd Dörken and collaboration partners

Activated RAS/BRAF oncogenes induce cellular senescence as a tumor suppressive barrier in early cancer development – as recently demonstrated by our group (Braig et al., Nature, 2005) – at least in part, *via* an oncogene-evoked DNA damage response (DDR). In contrast, Myc activation – although producing a DDR as well – is known to primarily elicit an apoptotic countermeasure. Using the Eμ-*myc* transgenic mouse lymphoma model, we found that both a cell-autonomous DDR-dependent and a non-cell-autonomous transforming growth factor– (TGF-β)-mediated induction of cellular senescence limits Myc-driven tumor development. While acute induction of Myc signaling is sufficient to produce lymphoma cell senescence *in vitro*, we identified TGF-β as the pivotal senescence trigger *in vivo*. We demonstrate that TGF-β is not secreted by lymphoma cells but is delivered by macrophages upon their activation by apoptotic lymphoma cells. These findings, detectable in human aggressive B-cell lymphomas as well, establish a novel network of heterotypic cell-cell interactions in which apoptotic tumor cells launch a paracrine response in non-malignant bystanders that limits tumorigenesis by cellular senescence.

Selected Publications

Bouchard*, C., S. Lee*, V. Paulus-Hock, C. Loddenkemper, M. Eilers and C.A. Schmitt. 2007. FoxO transcription factors suppress Myc-driven lymphomagenesis via direct activation of ARF. Genes Dev. 21: 2775-2787. *) equal contribution

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Thomas Sommer

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Mechanisms of Protein Quality Control

The endoplasmic reticulum (ER) is a cellular organelle through which a significant proportion of proteins pass on their way to their functional sites in membranes, exocytic and endocytic compartments, or the cell exterior. Far from being a passive traffic way, the ER is home to an array of molecular chaperones, which help proteins to fold and guide their maturation. Despite this support, 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. Malfunction or aggregation of defective proteins challenges the homeostasis of the ER and the cell as a whole. As a consequence, evolution has produced a protein quality control (PQC) network that operates on several levels to maintain the integrity of the ER.

The work of this group focuses on how the ER protein quality control system selectively disposes aberrant proteins without jeopardizing nascent polypeptides that also populate this compartment. Current data suggest that polypeptides are initially protected from degradation by a specific N-linked glycan structure to allow their maturation. Later, ER mannosidases generate a unique glycan code that flags potentially misfolded substrates. This signal is decoded by an ubiquitin ligase anchored in the ER membrane. Proteins committed for degradation are transported across the ER membrane in a process termed protein dislocation. Subsequently, substrate molecules are ubiquitylated and degraded by the 26S proteasome. This process is referred to as ER associated degradation or ERAD. But misfolded proteins are not the only substrates of this system. It also regulates sterol synthesis by eliminating the pathway's rate-limiting enzyme when sterols are abundantly available. This example shows that ERAD also has a regulatory component that may not be limited to this anabolic pathway.

Since the ERAD pathway appears to be conserved from yeast to mammals, we use the model organism *Saccharomyces cerevisiae* to investigate the fundamental mechanisms and to identify the key components of this important pathway. These are the HRD ubiquitin ligase and the Doa10 ubiquitin ligase. The HRD-ligase is crucial for turnover of membrane-bound (ERAD-M) and ER-luminal substrates (ERAD-L). Doa10 targets membrane proteins for degradation that carry lesions in their cytoplasmic domains (ERAD-C). Both yeast ubiquitin ligases and their co-factors that have been identified so far are summarized in Fig. 1. The mammalian counterparts of the yeast components are mentioned as well.

Usa1 Functions as a Scaffold of the HRD-ligase

Sabine Horn, Ernst Jarosch, Christian Hirsch in collaboration with Jennifer Hanna, Anja Schütz, and Udo Heinemann

The multiprotein HRD-ligase (HMG-CoA Reductase Degradation) singles out terminally misfolded proteins of the ER and routes them for degradation to cytoplas-

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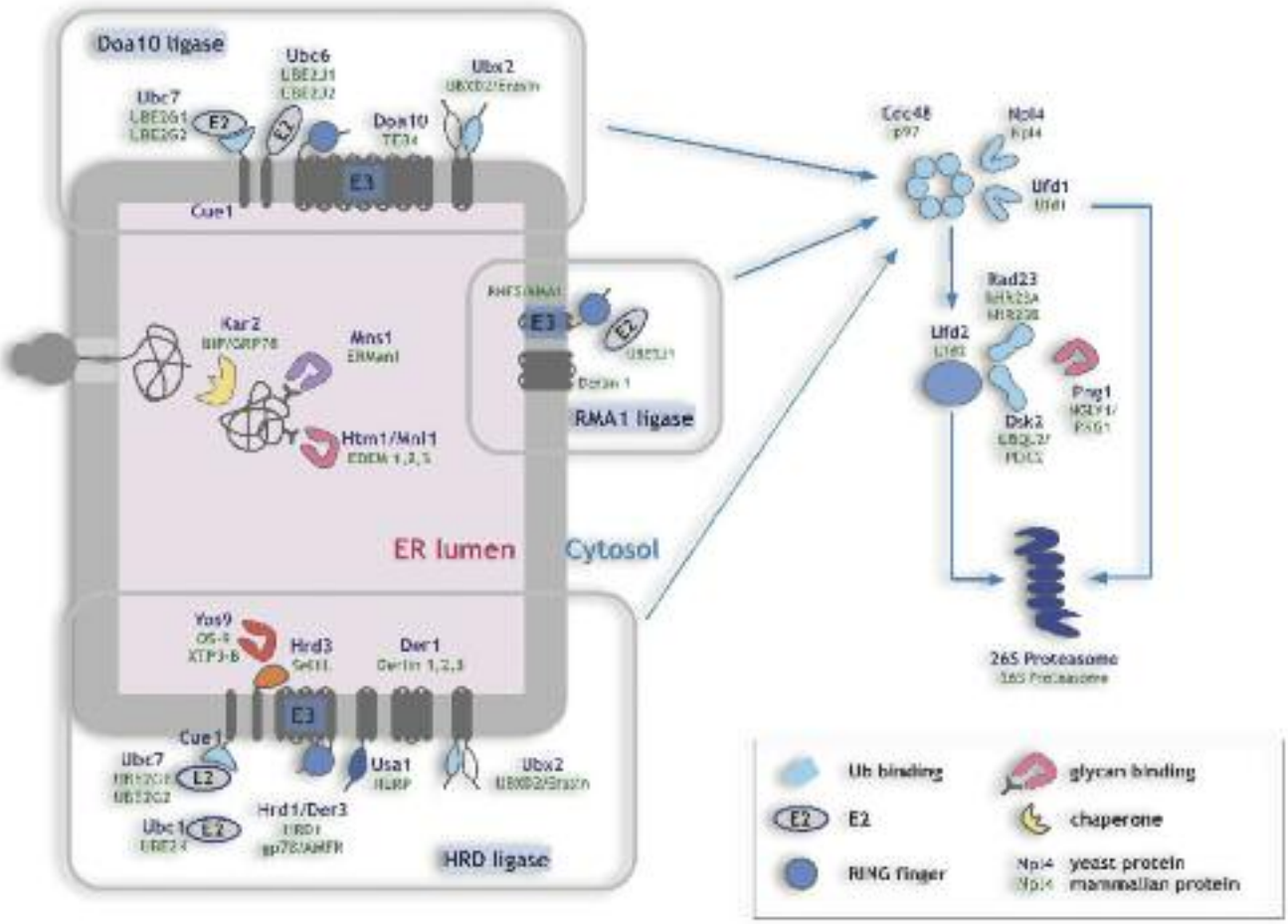

Figure 1. *ER-associated protein degradation in yeast and mammalian cells. Molecular chaperones and proteins of the glycosylhydrolase-47 family (Mns1 and Htm1) detect misfolded polypeptides and direct them to membrane bound ligases (Doa10, RMA1, HRD). After dislocation to the cytosolic face of the ER membrane, substrates are ubiquitylated by an ubiquitin ligase. All ligase complexes comprise a central, catalytic active RING finger protein (E3), ubiquitin-conjugating enzymes (E2), and additional factors. The AAA ATPase Cdc48 releases ubiquitylated molecules from the ER membrane. The adapter proteins Rad23 and Dsk2 escort the ubiquitylated substrates to the 26S proteasome for degradation. Concurrently Png1 deglycosylates glycoproteins through its association with Rad23. Proteins containing a glycan-interaction motif or ubiquitin-binding domains are depicted in red and blue, respectively. Proteins are labeled with their yeast names in blue and green letters indicate the mammalian counterpart.*

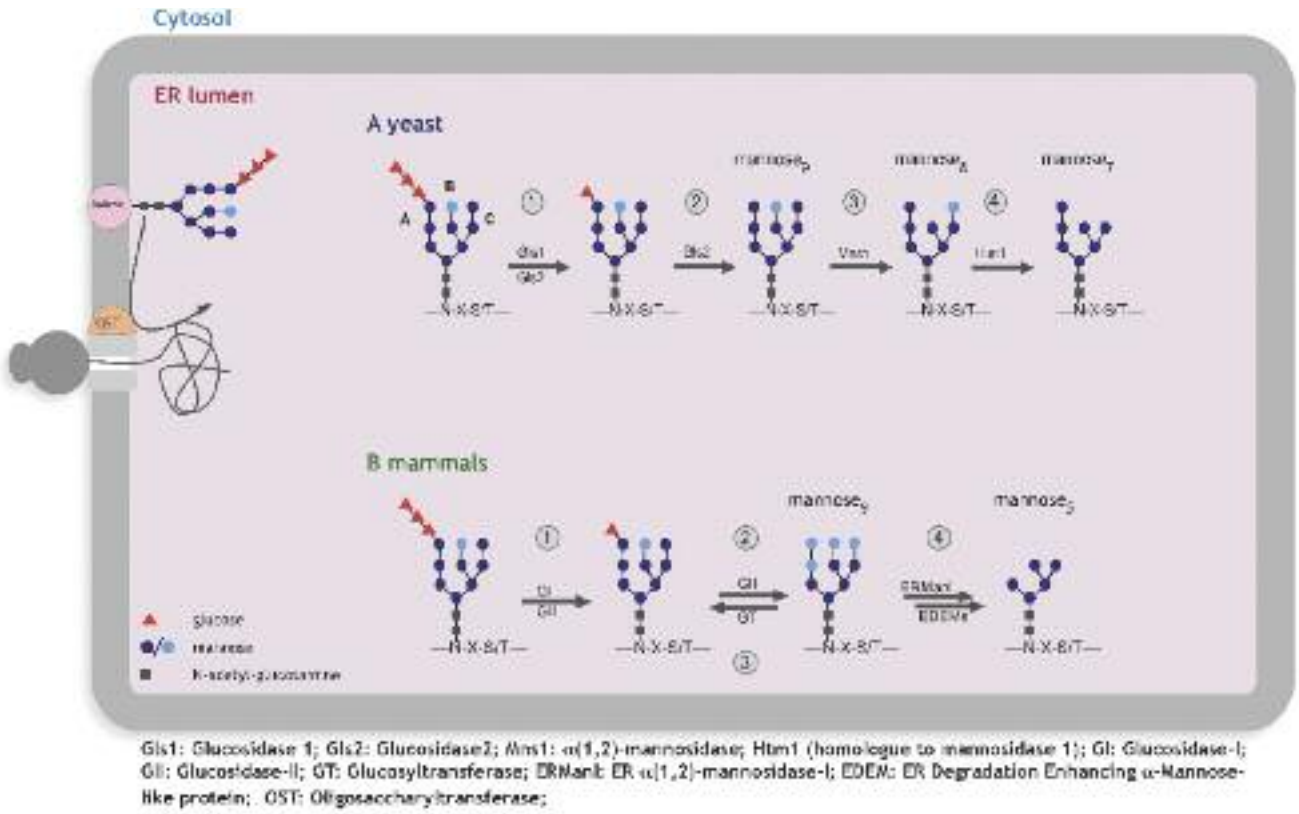

Figure 2. *Processing of N-linked glycans in the yeast and mammalian ER. (a) A glucose$_3$-mannose9-N-acetylglucosamine$_2$ oligosaccharide is initially attached to asparagine in NXS/T motifs by the oligsyccharyltransferase. Gls1 (glucosidase 1) and Gls2 remove the two outmost glucose residues and generate a glucose$_1$-mannose$_9$-N-acetylglucosamine$_2$ sugar (step 1). Further processing by Gls2 results in a mannose$_9$-N-acetylglucosamine$_2$ glycan that protects the glycoprotein from disposal (step 2). Mns1 trims the outmost mannose moiety of the B branch, yielding a mannose$_8$-N-actelyglucosamine$_2$ oligosaccharide, which indicates a protein that is retained in the ER for a prolonged period of time (step 3). Subsequently Htm1 processes the C-branch yielding a mannose7-N-actelyglucosamine2 oligosaccharide (step 4). (b) In contrast to yeast, in mammalian cells removal of the residual glucose residue from the A branch by glucosidase-II (GII, step 2) is reversible. GT reglucosylates glycoproteins that have not attained their native fold (step 3). Extensive demannosylation by ERManI and probably the EDEM proteins yield a mannose$_5$-N-acetylglucosamine$_2$ glycan that is a signal for disposal (step 4). Light blue mannose residues protect the glycoprotein from degradation until they are removed.*

mic 26S-proteasomes. Specific functions, like substrate selection and ubiquitylation, are assigned to distinct subunits of the HRD-ligase. For example substrate binding involves the ER-luminal domain of Hrd3 and the associated lectin Yos9. Ubiquitylation depends on the RING-finger protein Hrd1 and the ubiquitin-conjugating enzyme Ubc7 and its co-factor Cue1. The driving force for dislocation is provided by ATP hydrolysis at the hexameric Cdc48 (in mammals p97 or VCP) complex. It consists of Cdc48 itself and the associated co-factors Ufd1 and Npl4. This ATPase binds to Ubx2 and in addition to poly-ubiquitylated substrates at the ligases. Two additional subunits of the ligase complex have been identified, Der1 and Usa1, but their precise function is still under debate. It is furthermore speculated that the ligase forms a channel in the membrane through which substrates are dislocated. However, other channels have been discussed as well and direct evidence for the channel hypothesis is still missing.

By biochemical analyses we were now able to show that Usa1 plays a dual role within the HRD ligase complex: First, it recruits the ancillary factor Der1 and second it mediates oligomerization of the HRD ubiquitin-ligase. These separate activities mirror different requirements for the processing of malfolded proteins with distinct topology. On one hand, Usa1 promoted oligomerization of Hrd1 is predominantly required for the breakdown of membrane proteins while it is dispensable for the turnover of soluble polypeptides. On the other hand, Der1 recruitment, in turn, is a prerequisite only for the degradation of soluble substrates. In this collaborative work with the group of Udo

Heinemann, we could also identify the relevant domains of Usa1, which are involved in the relevant protein interactions at the ligase complex. The N-terminus of Usa1 binds the very C-terminal 40amino acids of Hrd1. Binding of Der1 involves the C-terminal region of Usa1. Thus, Usa1 functions as a scaffold that assembles the different activities of the HRD-ligase for processing of different classes substrates that differ in their topology. In a broader sense, our data strengthen the view that scaffold proteins modulate ubiquitin ligase activities rather then being passive devices.

Htm1 protein generates the N-glycan signal for glycoprotein degradation in the endoplasmic reticulum

Christian Hirsch in collaboration with Simone Clerc, Daniela Maria Oggier, Paola Deprez, Claude Jakob, and Markus Aebi

N-linked glycans are essential for the breakdown of glycoproteins. The covalently attached oligosaccharide structure is used as a signal to display the folding status of the protein. Newly synthesized proteins receive a Glc3Man9GlcNAc2 modification. Such a glycan structure protects a newly synthesized protein from degradation. Subsequently it is trimmed by glucosidases and mannosidases until a specific signal is generated, which is recognized by the quality control ubiquitin ligase. Since trimming of glycans is slow, these processing steps provides a time window in which a newly synthesized protein can adopt its cognate conformation.

In a collaborative effort we were able to define the function of Htm1 as an α1,2-specific exo-mannosidase that generates the Man7GlcNAc2 oligosaccharide with a terminal α1,6-linked mannosyl residue on degradation substrates. This oligosaccharide signal is decoded by the ER-localized lectin Yos9 that in conjunction with Hrd3 triggers the ubiquitin-proteasome dependent hydrolysis of these glycoproteins. The Htm1 exo-mannosidase activity requires processing of the N-glycan by glucosidase I, II and mannosidase I, resulting in a sequential order of specific N-glycan structures that reflect the folding status of the glycoprotein (Fig. 2).

Since Htm1 generates the crucial signal that flags a protein for degradation, its activity must be tightly controlled. Thus, we searched for associated factors that could be involved in controlling Htm1 activity. Surprisingly, we co-purified the protein disulfide isomerase Pdi1 together with Htm1. Binding of Pdi1 occurs at the Htm1 C-terminus whose function is unknown. Our results raise the sepculation that the activity of Htm1 is linked to incorrect disulfide bridge formation.

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Nuclear Signaling and Chromosomal Domains

Using *Drosophila* as a model our group investigates chromatin switches that are crucial for Notch and TGF-β signal transduction and mechanisms involved in compartmentalization of chromatin.

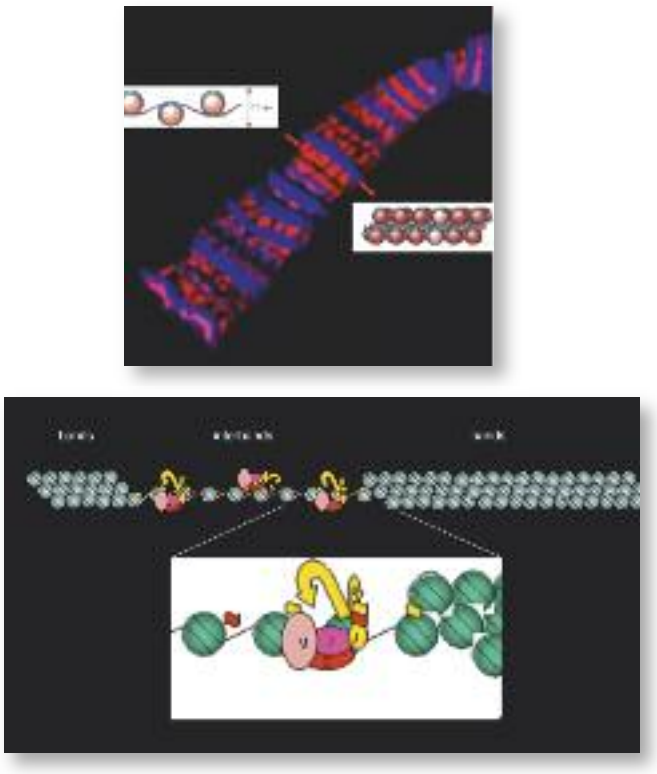
Skip/Bx42 dependent target gene and cell cycle regulation related to Notch signaling

On binding transmembrane ligands of adjacent cells the Notch receptor splits off its intracellular domain (Notch-IC) which migrates into the nucleus and transiently binds to its target genes. On binding Notch recruits an activator complex to genes that previously were repressed by a silencing complex bound to a chromatin platform provided by the conserved CSL proteins CBF1/Su(H)/Lag3. By exchanging the repressor complex for the Notch activator complex target genes become activated. However, neither the exact composition of the activator and the repressor complexes nor the mode of the switching mechanism are as yet known.

We previously characterized Bx42/SKIP a conserved chromatin co-regulator protein. As in *Drosophila* the mammalian homologue interacts with nuclear components of the Notch pathway like Notch-IC and Su(H)/CBF1. By induced Bx42-RNAi we demonstrated that these interactions are biologically important since the expression of several Notch target genes is dependent of the presence of Bx42/SKIP and tissue specific knock down of Bx42/SKIP results in *Notch*-like phenotypes. As a precondition for crystallographic studies we currently map the interaction sites of Bx42/SKIP with Notch-IC, Su(H) and the Hairless protein. For instance, in contrast to previous work by others we mapped the Skip interaction side on the C-term of Notch-IC downstream of the ankyrin repeats and the RAM domain. Work is in progress to investigate how the Skip/Notch-IC interaction is translated into the activation of target genes Expression of the most conserved part of Skip, the central SNW region, results in dominant negative phenotypes that are suppressed by over-expression of the FL Skip protein, by Notch gain of function mutations or putative Skip interacting proteins and enhanced by Notch loss of function alleles. Over-expression of SNW region in late larval eye discs results in a small eye phenotype by specific suppression of the Notch-dependent division of retinal precursor cells at the G2-M transition that is cyclin A dependent. Comparing the expression profile of the Bx42 dominant negative to wild type cells on microarrays we find that a restricted number of genes is affected by SNW over-expression, amongst them Dp, a dimerization partner of E2F and the chromatin repressor protein Sina. Interestingly, in mammalian cells Bx42/SKIP interacts with and counteracts the repressive effect of the Rb protein and shows an interaction with the E2F family of cell cycle regulators. The role of Skip and interacting proteins in cell cycle regulation will be investigated in more detail in *Drosophila* cell cultures

Chromatin domains and boundaries

Transgenes inserted into the mammalian genome regularly become silenced and occasionally hyperactivated at their site of insertion reflecting the impact of local chromatin structure. Interestingly, elements found at chromosomal domain boundaries shield for such effects by providing enhancer blockers or barrier ele-



Above: Tip of chromosom3L showing the Z4 in interbands red (IIF) and the DNA in blue. Inserts indicate the chromatid organisation in bands (30 nm fiber) and interbands (10 nm fiber) respectively. Below: Scheme of the Z4-complex with Chriz- (C), Z4- (Z), MBD-R2- (M) and other yet uncharacterized proteins that results in recruitment of the H3S10 kinase Jil-1 and H3S10 phosphorylation (yellow flag) followed by other chromatin modifications like H3K4 trimethylation (red flag).

ments that insulate nearby regulatory elements or the spreading of adjacent heterochromatin. Thus, besides for scientific curiosity, the knowledge of chromosomal domain structure and their boundaries is of immediate medical importance, in particular in gene therapy. Formation of boundaries is part of a concept, that eukaryotic genomes are organized into functionally independent chromosomal domains. A well known chromosomal domain is the globin domain in man, mouse and chicken whose boundary function essentially requires the CTCF protein. The *Drosophila* homologue dCTCF is found at a restricted number of interbands on polytene chromosomes and is required for boundary formation in homeotic gene clusters. It interacts with the protein CP190 present at band interband boundaries At some sites CP190 is essential for dCTCF chromosomal binding. dCTCF and CP190 binding was mapped genome-wide by ChIP on Chip experiments and found to be correlated to active promoters and insulators.

On *Drosophila* polytene chromosomes chromosomal domains differing in their degree of condensation become apparent as a conserved pattern of dark bands (chromatids as $\geq$30 nm fibers) and light interbands (chromatids as ~10 nm fibers). The pattern is conserved between tissues, suggesting a constitutive chromosomal domain organisation established and maintained by chromatin boundaries. By localization, isolating and characterizing DNA sequences from band/interband units, their associated proteins and histone modifications we try to understand the process of chromosomal domain formation. Mutation of the interband specific zinc finger protein Z4 results in a dramatic loss of band/interband structure, presumably by affecting the maintenance of chromosomal boundaries. The Z4 protein is complexed with other interband proteins like those organizing dosage compensation on the X chromosomes and the histone kinase Jil-1 [Gan PhD thesis Humboldt University 2009]. Tethering Jil-1 kinase in chromatin results in local decondensation and Z4-mutations affect interband specific histone H3S10 phosphorylation and H3K4 trimethylation. Z4 interacts in a protein complex with the novel chromodomain proteins Chriz and MBD-R2. Chriz is required for Jil-1 and Z4 binding, H3S10 interphase phosphorylation and the maintenance of chromosome structure [Gan PhD thesis Humboldt University 2009]. The DNA region required for Z4/Chriz complex binding was mapped on polytene chromosomes by high resolution in situ hybridization and by ChIP on chromatin of diploid cells we could show that the complex binds to the same region in these cells too, suggesting its general role in a constituting a chromatin structure that is shared between cells of different tissues.

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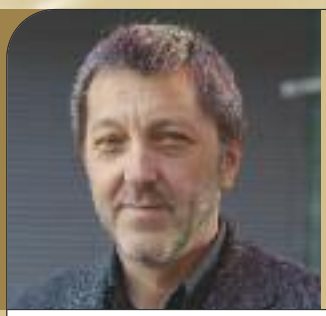

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Macromolecular Structure and Interaction

Inside cells the interplay of large and small molecules is responsible for the catalysis of chemical reactions, the regulation of gene expression and the transport of proteins and other cargo to their sub-cellular destinations. These activities depend on the fine details of macromolecular interactions which are amenable to structural characterization at the atomic level. We are combining the powerful methodology of X-ray crystallography with biochemical and biophysical studies of proteins, nucleic acids, carbohydrates, lipids, membranes and small metabolites to analyze their structures and interactions. The results of these studies have yielded insight into the architecture and glycosyl hydrolase activity of bacteriophage tailspike proteins, the cooperation of transcriptional regulators in controlling gene expression in the conjugative RP4 plasmid, and structural aspects of vesicle tethering to the Golgi apparatus. Our work is greatly facilitated by privileged access to the synchrotron storage ring BESSY in Berlin and the Helmholtz Protein Sample Production Facility.

Tailspike endoglycosidases

Tailed bacteriophages consist of an icosahedral head filled with double-stranded DNA and a portal channel with tail machine. In the P22 phage, 12 subunits of the gp1 protein form the portal structure which serves as the base for the association of 39 additional subunits of four different proteins, gp4, gp9, gp10 and gp26. The six tailspike proteins (TSP, gp9 of P22) are thought to establish physical contact with the bacterial host cell after degrading the cell-surface glycans.

The high-resolution crystal structures of phage Sf6 TSP and phage HK620 TSP show a conserved tripartite architecture of these proteins with an α-helical N-terminal region, a large right-handed β-helix and a β-sheet C-terminal domain. The central β-helix bears distant structural similarity to cross-β structures known to exist in amyloid deposits. Quite surprisingly, the crystallographic and mutational analysis revealed the location of the Sf6 endorhamnosidase active site in a cleft between the subunits of the trimeric TSP, whereas single subunits of both P22 and HK620 are capable of glycan hydrolysis. The C-terminal domain of Sf6 TSP shares a conserved fold with viral capsid proteins, suggesting a common evolutionary origin of capsid and tail components. Up until recently, all phage TSP were crystallized in the absence of their N-terminal domains which attaches them to the base of the tail machine through gp10 and gp4. We have now designed a P22 TSP mutant that yields crystals and will permit the fitting of an intact tailspike protein structure to high-resolution cryo-electron microscopic images of the tail machine.

Nucleic acid-interacting proteins

Transcriptional regulators have to recognize specific sequence motifs in double-stranded DNA. Their activity

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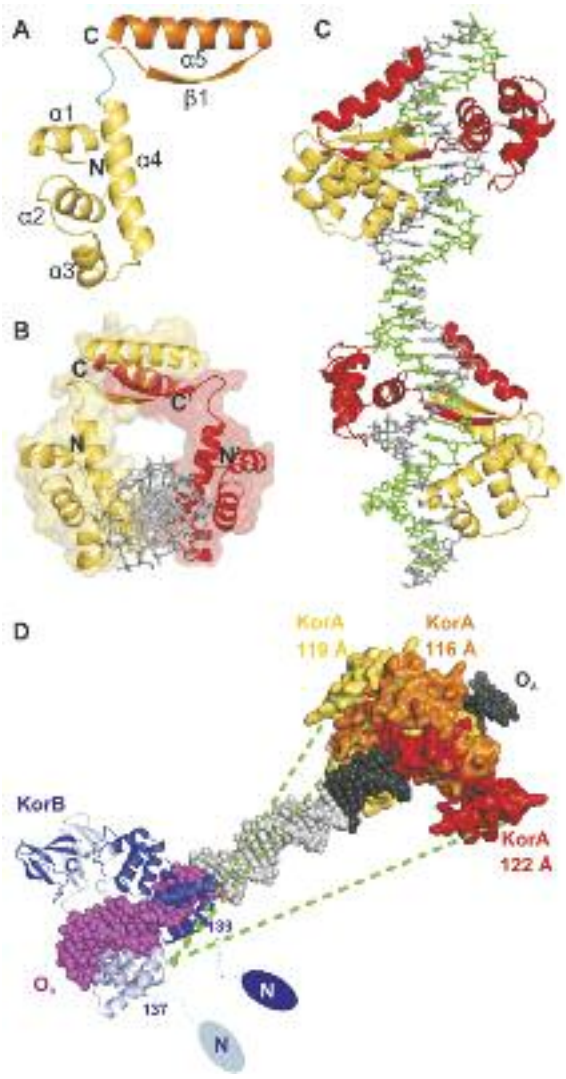

Figure 1. ***Co-operation of transcriptional regulators in RP4 gene expression.*** *(A) Crystal structure of the RP4-encoded repressor KorA. The DNA-binding domain including a classical helix-turn-helix motif is colored yellow, the dimerization region orange. (B) Dimerization and DNA binding of KorA. The two subunits of the dimer are colored in yellow and red. The view is along the DNA double helix. (C) End-to-end stacking of two 18-bp DNA fragments bound to KorA dimers in crystals of the KorA-DNA complex. The operator sequence is recognized through specific hydrogen bonding of protein sidechains from the recognition helix to basepairs in the major groove of the DNA. The two strands of the double helix are colored green and gray. (D) Model for the KorA-KorB interaction in RP4 gene regulation. The flexible N-terminal domains of KorB are predicted to contact the conserved dimerization domain of KorA over a distance which remains nearly the same for operator midpoints at 32, 33 or 36 bp distance, because each basepair added to the spacer DNA rotates the contact regions towards each other while increasing the lateral separation.*

is modulated by binding to small molecules, co-activator or co-repressor proteins. We are studying transcriptional repressor co-operation in a simple model system, the conjugative plasmid RP4. Genes encoded on this plasmid are under the control of two global repressors, KorA and KorB, that recognize multiple bindings sites each, O_A and O_B. At five promoter regions of RP4, an O_A site is near an O_B site, and at four of these promoters the center-to-center distance of these operators is between 32 and 36 basepairs. In earlier work, we had determined the crystal structure of a central domain of KorB, KorB-O, bound to double-stranded DNA containing the O_B1 sequence. KorB was characterized as a protein containing natively unfolded regions near its N-terminus and in a segment connecting KorB-O with the C-terminal dimerization domain, KorB-C.

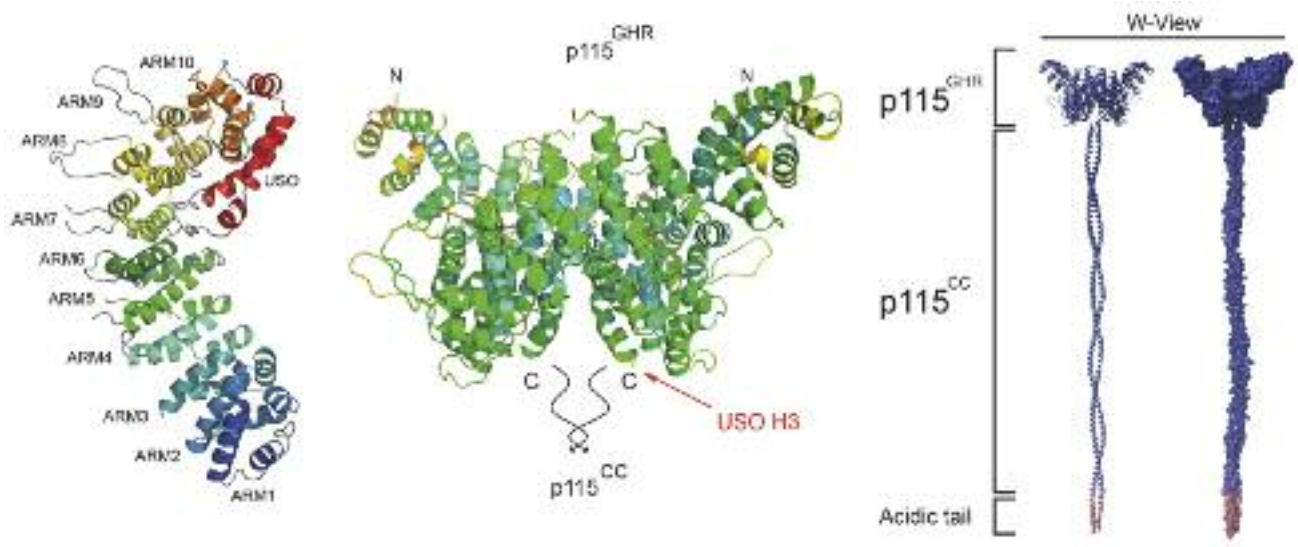

Figure 2. *Crystal structure of the human golgin p115. The globular head region of p115, p115^{GHR}, is composed of nine canonical armadillo repeats (ARM1 to ARM9) and an unusual repeat, ARM10, whose additional α-helix (USO) folds into the concave surface of p115 (left). In the crystal, p115^{GHR} is in a dimeric arrangement with C-termini in close vicinity (center). A model of intact p115 containing the coiled-coil and acidic C-terminal regions (right) closely resembles electron micrographs of human p115 and the homologous yeast Uso1p.*

The crystal structure of KorA bound to an 18-basepair DNA fragment containing a consensus O_A site revealed a classical binding mode in which sidechains from the recognition helix of the KorA helix-turn-helix motif make specific hydrogen-bonded contacts to basepairs in the DNA major groove. The dimerization domain of of KorA has a sequence that is conserved in the homologous RP4-encoded proteins TrbA and KlcB. Based on the crystal structures of DNA-bound KorA and KorB we propose a model for repressor co-operation in RP4 gene regulation in which a contact between the flexible N-terminus of KorB with the conserved dimerization domain of KorA or TrbA is postulated (Figure 1). According to this model, the flexibility of KorB serves to measure the distance between operators along the DNA while allowing for different spatial dispositions of the contact sites on KorA or TrbA caused by the double-helix twist.

Vesicular transport to the Golgi apparatus

Vesicular transport in eukaryotic cells depends on conserved sets of proteins involved in the sequential steps of vesicle budding, uncoating, and tethering to the target membrane, as well as in membrane fusion and cargo release. Tethering, the process in which a first physical contact between vesicle and target membrane is established, is regulated by a small GTPase belonging to the Rab/Ypt family and involves both heteromultimeric tethering complexes and factors characterized by extended coiled-coil regions. Vesicle tethering to the Golgi apparatus of human cells thus involves the Rab1 GTPase, the transport protein particle (TRAPP) consisting of at least seven different subunits and p115 or other coiled-coil containing proteins of the golgin family. We are taking a combined biochemical and structural approach to studying the role of these molecules in

vesicle transport to the Golgi. Our earlier work has focussed on subunits and sub-complexes of TRAPP

Recently, the golgin p115 was studied by X-ray diffraction methods. The crystallized protein fragment comprised the globular head region ($p115^{GHR}$) and lacked 50 N-terminal residues as well as the coiled-coil and acidic tail regions of p115. The crystal structure reveals an armadillo fold of $p115^{GHR}$ with nine canonical and one unusual, C-terminal repeat (Figure 2). This C-terminal repeat contains an extra α-helix which folds back into the concave surface of the armadillo region to give the C-terminal half of the armadillo domain a compact, globular appearance. In the crystal, two $p115^{GHR}$ molecules are arranged to form a symmetric dimer. A model for an intact p115 based on this dimeric arrangement in combination with a coiled-coil region of appropriate length agrees well with electron microscopic images of p115 and its yeast homolog, Uso1p. The crystal structure of $p115^{GHR}$ reveals important determinants of this golgin's interaction with components of the COP I vesicle coat, the conserved oligomeric Golgi (COG) complex and the Rab1a GTPase in vesicular transport at the Golgi.

Protein Sample Production Facility

Our group at MDC closely co-operates with the Helmholtz-Zentrum für Infektionsforschung (HZI) in Braunschweig in the framework of the Helmholtz Protein Sample Production Facility (Helmholtz PSPF). The PSPF offers expertise in protein production for structural biology using various host systems tailored to specific experimental requirements. A reliable methodology for the production of crystallizable protein samples in bacterial, insect or mammalian cells has been established since the start of the PSPF, where the Berlin group focusses on parallelized expression cloning and protein production using *Escherichia coli* whereas the group at HZI is developing methods that rely on eukaryotic cell culture systems.

Recently, structural biologists have increasingly been looking past single protein molecules and turned their attention to structural studies of firm or transient protein interactions. Various approaches to the production and characterization of protein complexes for structural studies have been evaluated and implemented. These techniques include a set of vectors allowing the co-expression of an, in principle, unlimited number of protein-coding genes in *E. coli* and the analysis of protein-protein and other protein-ligand interactions in thermofluor assays.

Selected Publications

Barbirz, S, Müller, JJ, Uetrecht, C, Clark, AJ, Heinemann, U. Seckler, R (2008). Crystal structure of *Escherichia coli* phage HK620 tailspike: Podoviral tailspike endoglycosidase modules are evolutionarily related. Mol. Microbiol. 69, 303-316.

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Structure and Mechanism of Membrane-remodeling G-proteins

Guanine nucleotide binding proteins (G-proteins) are involved in a diverse range of cellular processes including protein synthesis, sensual perception, vesicular transport and signal transduction cascades. Whereas small G-proteins are molecular switches that cycle between an active GTP-bound form and an inactive GDP-bound form, large G-proteins of the dynamin superfamily are mechano-chemical enzymes that use the energy of GTP hydrolysis to actively remodel membranes. Members of both groups bind to membranes, and this interaction is crucial for their function. Our projects aim to elucidate the interaction and reciprocal modulation of membranes and G-proteins using structural, biochemical and cell-biological methods.

1. EHD as a molecular model for membrane remodelling G-Proteins

Members of the dynamin superfamily are multi-domain proteins with an amino-terminal G-domain. Its founding member dynamin oligomerises around the neck of clathrin-coated vesicles and induces vesicle scission in a reaction which is dependent on GTP hydrolysis. The mechanism of vesicle scission is poorly understood at the molecular level.

In this project, we want to establish the less characterised EHD family as a model system to understand principles of membrane remodelling in the dynamin superfamily. EHDs comprise a highly conserved eukaryotic protein family with four members (EHD1-4) in mammals and a single member in *C. elegans* and *D. melanogaster*. The proteins are built of an amino-terminal G-domain, followed by a helical domain and a carboxy-terminal EH-domain known to interact with linear peptide motifs of proteins involved in endocytosis. EHDs can be found at vesicular and tubular structures *in vivo,* and EHD family members have been shown to regulate several trafficking pathways including the exit of cargo proteins from the endocytic recycling compartment.

We could show that EHD2 binds with low affinity to nucleotides, like other members of the dynamin superfamily. Surprisingly, ATP rather than GTP was bound. We demonstrated that EHD2 could also bind to negatively charged liposomes, and this binding resulted in the deformation of the liposomes into long tubular structures around which EHD2 oligomerised in ring-like oligomers (Figure 1a). Furthermore, the slow ATPase activity of EHD2 was enhanced in the presence of liposomes, which is another typical feature of dynamin-related proteins.

We solved the crystal structure of an EHD2 dimer in the presence of a non-hydrolysable ATP analogue (Figure 1b). Dimerisation is mediated via a highly conserved surface patch in the G-domain. We could show that the lipid-binding sites in each dimer are located at the tip of the helical domains and create a highly curved lipid interaction site, which might contribute to the membrane remodelling activity of EHD2. We further predicted the architecture of the EHD2 oligomeric ring where 20 EHD2 dimers assemble via the G-domain to form a tightly packed oligomer (Figure 1c).

Having solved the structure, we will continue this project to understand the structural changes associated

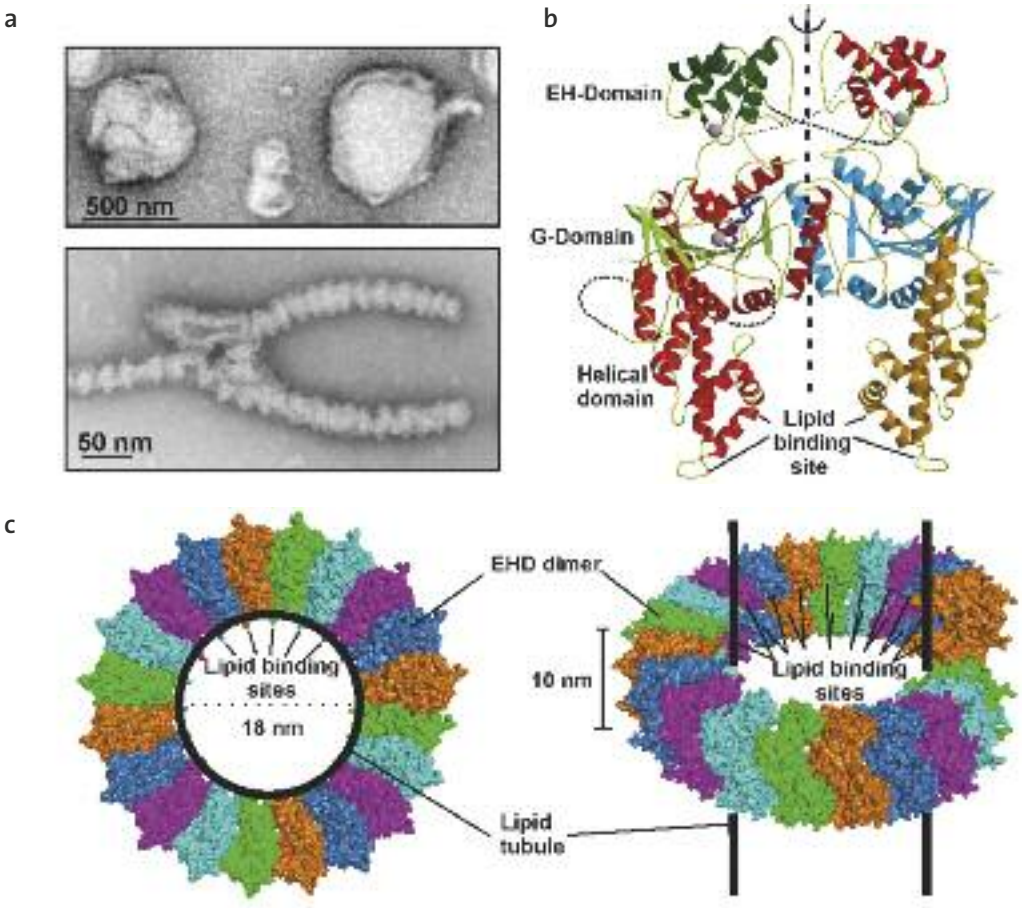

Figure 1a: *EHD2 is tubulating phosphatidyl-serine (PS) liposomes and oligomerises in ring-like oligomers as analysed by negative-stain electron microscopy. Micrographs of PS liposomes in the absence (top) or presence (bottom) of EHD2 and 1 mM ATP-γ-S.*

Figure 1b: *Ribbon-type presentation of the EHD2 dimer. The structure of EHD2 was determined by X-ray crystallography. The two-fold axis is indicted by a dashed line.*

Figure 1c: *Top and side view of the proposed EHD2 oligomer with the lipid-binding sites of EHD2 pointing towards the liposome surface. The EH-domains are omitted for clarity.*

with ATP binding and hydrolysis. Furthermore, we would like to obtain structural information of membrane-bound oligomerised EHD2 using electron-paramagnetic resonance studies. Finally, we are interested to study the physiological function of EHD2 using cellular studies.

2. Structure and function of the GIMAP family

The GIMAP GTPases comprise seven members in humans which are predominantly expressed in cells of the immune system. Some of the members localise to the mitochondrial membrane and are proposed to regulate apoptosis by regulating the entry of cytochrome *c* from the mitochondria into the cytosol. We will clarify the exact function of this protein family at the mitochondria and the interaction with membranes using structural, biochemical and cell-biological methods. These results will have implications for several types of leukaemia in which GIMAP members are overexpressed.

3. Structural insights into the antiviral effector MxA.

Mx (myxovirus-resistance) proteins are interferon-induced key effector molecules in the innate immune system mediating cellular resistance against a wide range of pathogens including influenza virus. The proteins belong to the dynamin superfamily and have been shown to tubulate liposomes and oligomerise in rings around the tubulated liposomes. We are interested to understand the structural details of oligomerisation and to decipher the exact mode of antiviral action using a structural approach.

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Bioethics and Science Communication

The group Bioethics and Science Communication was established in 2002. We analyze ethical, scientific, and social aspects of new health technologies. Areas of conflict concerning future developments of these technologies are disclosed by the comparison of scientific expectations with the ongoing social change.

Our research focuses on prerequisites and practical questions for communicating scientific challenges and results to the public in a procedure without preempting results, facilitating thus an open, informed, and fair discourse.

In particular, we are focusing on the evaluation of scientific uncertainties in health technology assessment by omics technologies, risk perception and risk communication, lay-expert relationships, and options for decision making.

Active participation in health technology assessment by different stakeholders including experts and lay people is crucial to our research. By developing and evaluating rational approaches, we aim at contributing to new forms of governance and deliberative democracy.

Relevant Topics:

ethical, legal and social aspects (ELSA) and assessments of biomedical technologies, especially of (1) stem cell research and (2) radiofrequency electromagnetic fields (RF-EMF) and cancer.

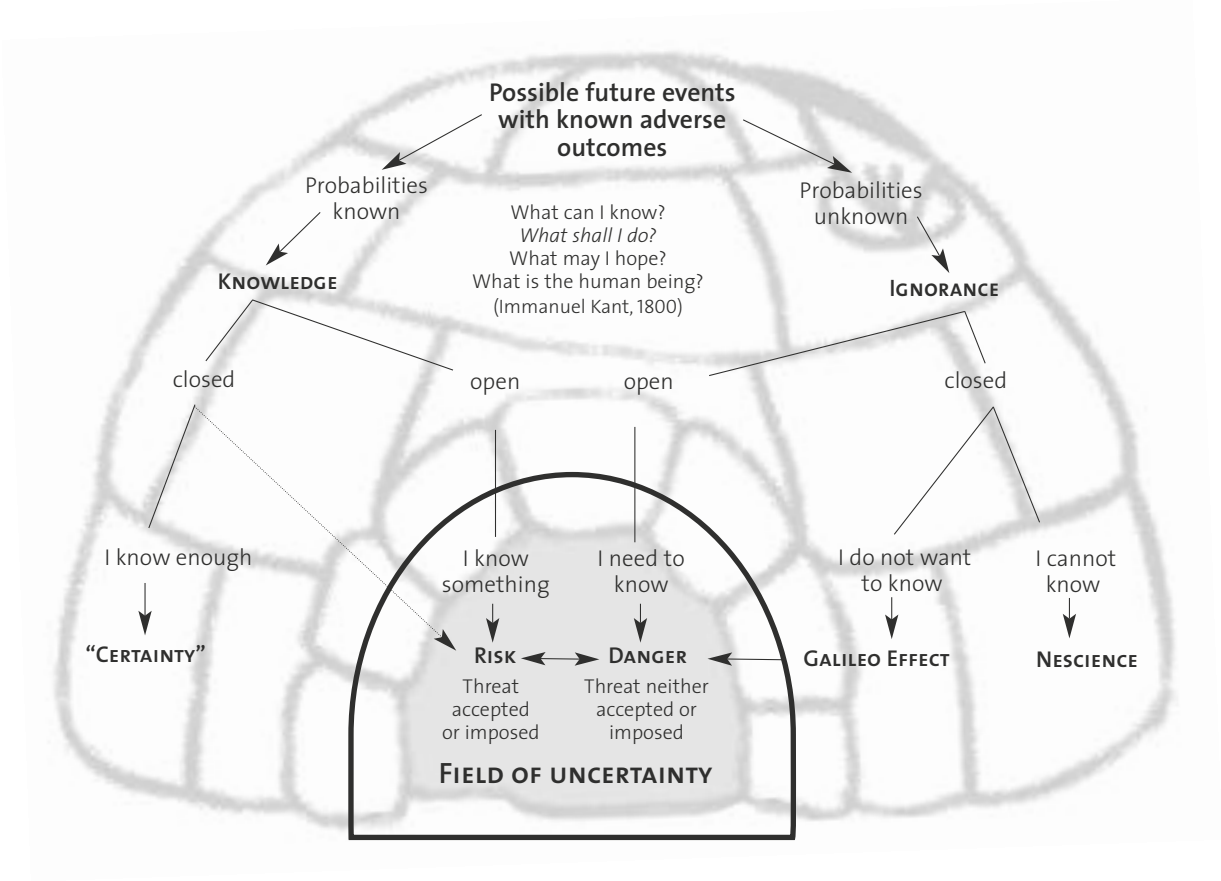
IMBA – Implications of Biomedicine for the Assessment of Human Health Risks

The project analyzes how toxicogenomics will transform the present risk management framework for biomedicine. The focus of the research is on the impact of risk characterization, risk perception, and risk communication concerning RF-EMF and cancer. IMBA is a project of the "Strategy Group Systems Analysis and Technology Assessment in the Helmholtz Association" (HGF SO-033 System Analysis). It started in June 2006 and will be completed in December 2009. The project:

- involves research groups and programmes inside the HGF, in close collaboration with German universities as well with international centers of excellence and adds critical mass to social science research on the impacts of toxicogenomic-based risk assessment by interdisciplinary collaboration between life science and social science
- contributes to the public debate on the impact of toxicogenomics on risk assessment through dialogue with various stakeholders
- fosters international collaboration through international networks and conferences.

SET-DEV – Science, Ethics and Technological Responsibility in Developing and Emerging Countries

The project SET-DEV aims in supporting the research systems of developing countries and countries with emerging economies, respectively, and especially those countries who are interested in developing their own ethical perspective in a more general context of sharing



As an outcome of the IMBA project, our group created the so-called "Igloo of Uncertainty", an epistemic topology for the term in question as well as a taxonomy of uncertainty (see also http://en.wikipedia.org/wiki/Uncertainty and Tannert et al., 2007).

responsibility for scientific and technological research with all involved parties. The SET-DEV project uses international dialogue, to contribute to capacity building in developing countries and emerging economies in the field of ethics and science. Our group incorporates the specific aspects of bioethics into this international EU project.

The project is structured in three interconnected stages:

- development by a comparison between networks of European and non-European researchers, including those working in emerging and developing countries (technological responsibility);
- implementation of a pilot programme, centred on actions of public dialogue, capacity building and training on the themes of ethics and technological responsibility;
- definition and dissemination of policy guidelines for strengthening the ethical orientation of research systems.

Internet presence

The website www.bioethik-diskurs.de with interactive discourse-pages, and the Online-Game GenEthix registers about 7,000 site visitors per month (May 2009).

Selected Publications

Elvers, HD, Jandrig, B, Grummich, K, Tannert, C. (2009). Mobile phones and health: Media coverage study of German newspapers on possible health effects of mobile phone use. Health Risk Soc. 11, 165-179.

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Tannert, C. (2007). The autonomy axiom and the cloning of humans. Hum. Reproduct. Genet. Ethics 13, 4-7.

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Differentiation and Growth Control in Lymphocyte Development and Immunopathogenesis

Chemokines are essential regulators of lymphocyte migration throughout the body. 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. In addition, chemokines are critical factors for the development and organization of secondary lymphoid organs. Our main focus is the role of homeostatic chemokine receptors like CXCR5 and CCR7 in lymphoid organ development, systemic immune responses, and chronic inflammatory diseases. In addition, we are interested in the immune modulatory and growth-inducing functions of chemokine receptors encoded by human herpesviruses, and the function of sphingophospholipid receptors in the immune system.

CCR7 mediates homeostatic lymphocyte recirculation and mucosal immunity

The homeostatic chemokine receptor CCR7 controls not only lymphocyte trafficking to and within secondary lymphoid organs, but also homeostatic migration of T and B lymphocytes through non-lymphoid peripheral tissues. CCR7 deficiency results in a massive accumulation of T- and B-lymphocytes in the peritoneal cavity. Mechanistically, the increase in the number of peritoneal lymphocytes is caused by an impaired egress of CCR7-deficient lymphocytes from body cavities. Most interestingly, a disturbed peripheral recirculation of lymphocytes also resulted in the development of ectopic lymphoid-like follicles and age-dependent histopathological changes in the gastrointestinal tract of CCR7-deficient mice. Since the formation of ectopic follicles precedes the development of epithelial histomorphological changes, we suggest that crosstalk between lymphoid aggregates and their adjacent epithelial tissue might contribute to the development of hypertrophic gastropathy. The underlying molecular mechanisms have been addressed by performing expression profiling of differentially expressed genes between wild-type epithelial tissue and CCR7$^{-/-}$ epithelial tissue or mucosal lymphoid aggregates. Several transcription factors and signaling molecules were selected on basis of the microarray data and are further characterized as potential mediators involved in this cellular crosstalk.

CXCR5-dependent autoantigen-driven lymphoid neo-genesis in a 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

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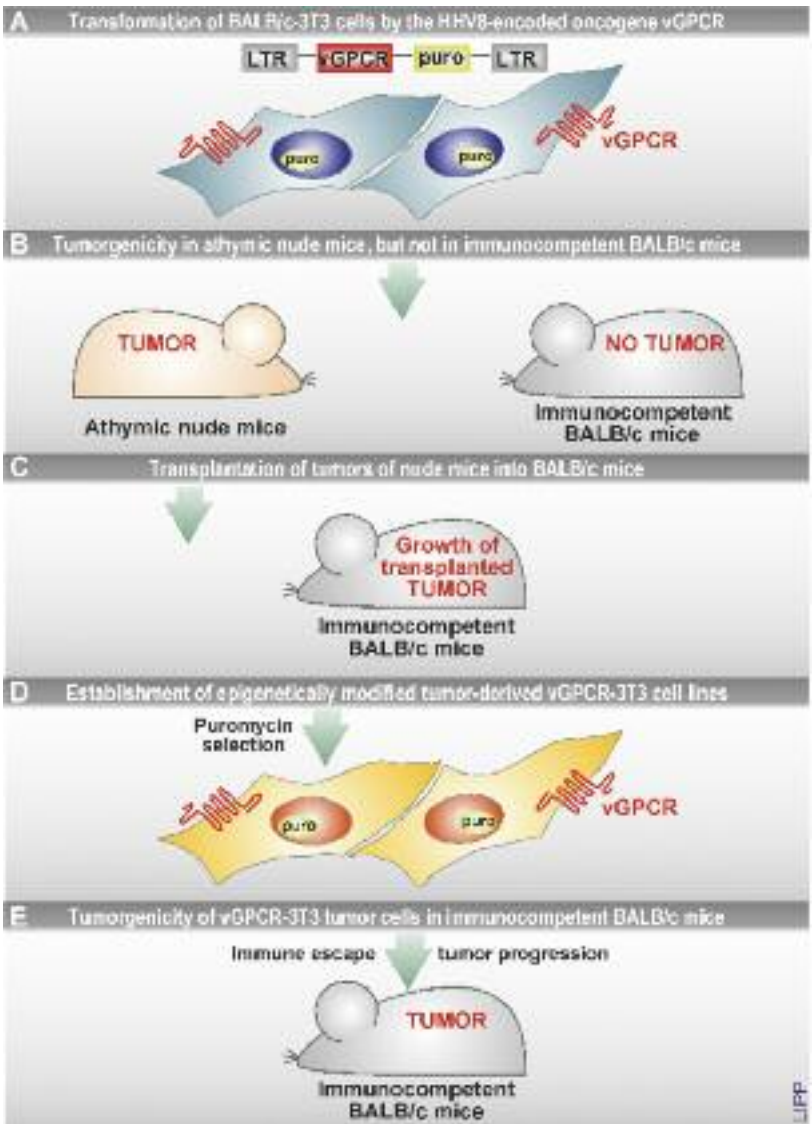
synovium of diarthrodial joints leads to irreversible joint damage, which results in chronic pain and disability. A characteristic feature for RA is the infiltration of the synovial tissue by granulocytes and large numbers of mononuclear cells. Another hallmark is the development of self-reactive T and B cells, leading to autoantibody production. We have developed a novel combined mouse model of collagen- and antigen-induced arthritis showing specific features of the chronic phase in human disease. The model is characterized by a rapid formation of ectopic lymphoid follicles as well as antibodies directed against collagen type II and citrullinated peptides (CCP). CCP-specific antibodies have a high prognostic value in humans and have so far not been described in mouse models of arthritis. However, the processes leading to chronic inflammation and the function of complex lymphoid microstructures have not been characterized so far. In this context, we are characterizing the role of cytotoxic T lymphocytes (CTLs) and follicular B helper T (T_{FH}) cells, which induce the differentiation of B cells into (auto) antibody-producing plasma cells. The development and organization of these ectopic structures were severely impaired in CXCR5-deficient mice proving its critical role as a signaling molecule in lymphoid neo-genesis during chronic inflammatory autoimmune diseases. Our results reinforce the link between chronic inflammation and the generation of tertiary lymphoid tissue at extra-nodal sites, which in turn drives local self-antigen-dependent interaction of memory/effector B and T lymphocytes resulting in aberrant chronic autoreactive immune responses.

Functional role of CXCR5 in the pathogenesis of *H. pylori*-induced chronic inflammatory gastritis

A potential relationship between chronic inflammation, establishment of extranodal tertiary follicles and lymphoma pathogenesis has been inferred from gastric MALT lymphomas in humans. In particular, expression of CXCL13, the ligand for CXCR5, has been associated with the formation of ectopic lymphoid follicles and the development of MALT-lymphoma in *H. pylori*-infected patients. We have analyzed the role of the homeostatic chemokine receptor CXCR5 in the formation of mucosal tertiary lymphoid tissue after *H. pylori* infection. CXCR5-deficient mice failed to develop lymphoid aggregates in the glandular stomach and exhibited lower *H. pylori*-specific serum IgG responses compared to infected wild-type mice. Thus, the development of mucosal tertiary ectopic follicles during chronic *H. pylori* infection is strongly dependent on the CXCL13/CXCR5 signaling axis.

Transcriptional control of follicular B helper T cell (T_{FH}) differentiation

Humoral immunity relies on B cell differentiation into antibody-secreting plasma cells. Follicular T helper (T_{FH}) cells, a specialized subset of effector T cells, are indispensable for the generation of these plasma cells, by providing B cell help during the germinal center (GC) reaction. T_{FH} cells supply signals for the survival, affinity maturation, isotype switching and immunoglobulin secretion of GC B cells and are thus important players in the generation of long-lived, high-affinity antibody-secreting plasma cells as well as memory B cells. Still, the molecular mechanisms through which naïve CD4 T cells become T_{FH} cells are largely unknown. The project aims at the identification of critical checkpoints in the differentiation of T_{FH} cells in order to better understand the development of adaptive immune responses. Large-scale gene expression analysis of human and murine T_{FH} cells as well as other CD4 T cell populations revealed unique expression patterns for transcriptional regulators and signaling molecules belonging e.g. to the delta notch signaling pathway. Our data suggests that T_{FH} cells constitute a separate lineage of T helper cells with a discrete developmental program and distinguishable effector function. As cell specialization is often



A novel animal model to study the role of epigenetic mechanisms in immune escape and tumor progression

A: BALB/c-3T3 fibroblasts have been transformed by retroviral transduction of the human herpervirus 8-encoded oncogenic chemokine receptor vGPCR. B: vGPCR-transformed BALB/c-3T3 fibroblasts that are tumorigenic in BALB/c nude mice, but as expected fail to induce tumors in their immunocompetent BALB/c counterparts. C: Tumor fragments obtained from nude mice, which comprise stroma cells of the tumor microenvironment, grow progressively in immunocompetent BALB/c mice. D: vGPCR-expressing BALB/c cell lines have been established from grafted tumor fragments under puromycin selection to get rid of any host-derived cells. E: Unexpectedly, the tumor-derived vGPCR-BALB/c cells gave rise to progressively growing tumors in immunocompetent BALB/c mice (Thirunarayanan et al., Oncogene 14:523, 2007). Ongoing experiments support our hypothesis that the passage of vGPCR-3T3 cells trough nude and immunocompetent mice leads to the induction of chromatin remodeling and epigenetic changes in the tumor-derived cell lines resulting in immune escape and progressive tumorigenesis.

associated with epigenetic changes in chromatin structure we are performing genome-wide ChiP-on-Chip analyses to define the landscape of activating/repressing histone modifications during T_{FH} cell differentiation in order to identify novel regulatory elements that may not be readily discernible through expression analysis and comparative genomics.

The human herpesvirus 8 encoded chemokine receptor vGPCR triggers progressive tumorigenicity of fibroblasts in immunocompetent BALB/c mice: A novel animal model to study the role of epigenetic mechanisms in immune escape and tumor progression

The human herpes virus 8 (HHV-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-expressing 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 immunocompetent BALB/c mice. Unexpectedly, vGPCR-expressing cells established from grafted tumor fragments gave rise to tumors in immunocompetent mice. These tumors exhibit a striking histological resemblance to KS including plump spindle cell morphology, a high degree of vascularization and brisk mitotic activity. Short interfering RNA directed at vGPCR abrogated or significantly delayed tumorigenesis of tumor-derived cells in nude mice, demonstrating that the tumor development is specifically driven by the vGPCR oncogene, but not by other

successive oncogenic mutations. We have now compared gene expression profiles of vGPCR-induced tumors in immunocompetent and nude mice and cell lines established from tumors in immuncompetent mice to the profiles of parental BALB/c-3T3 and vGPCR-transformed BALB/c-3T3 cells. This approach, in combination with with ChIP-on-Chip analyses, led to the identification of genes regulated by epigenetic modifications related to the successive passage of vGPCR-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 resulting in immune escape and progressive tumorigenesis.

Role of sphingophospholipid receptors in the immune system

The group of sphingosine-1-phosphate (S1P) receptors comprises five G protein-coupled receptors mediating a wide variety of biological functions. In order to characterize the as yet unidentified *in vivo* function of the $S1P_4$ receptor that was initially described in our laboratory, we have created and analysed two different $S1P_4$-deficient mouse models, including a lacZ knock-in reporter strain. The phenotype of these $S1P_4$-deficient animals suggest a role of $S1P_4$ in megakaryocyte maturation as well as in T cell biology. The biological behavior of $S1P_4$-deficient lymphocytes suggests furthermore an intricate interaction of the two S1P receptors predominantly expressed on lymphocytes, $S1P_1$ and $S1P_4$. Our results show for the first time an *in vivo* function of the $S1P_4$ receptor. Our results identify the $S1P_4$ receptor as a potential therapeutic target in clinical situation where an increased generation of platelets is required (i.e. massive bleeding) or, at the opposite, reactive thrombocytosis is to be prevented (i.e. thromobogenic clinical settings).

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Wengner, AM, Höpken, UE, Petrow, PK, Hartmann, S, Schurigt, U, Bräuer, R, and Lipp, M. (2007) CXCR5- and CCR7-dependent lymphoid neo-genesis in a murine model of chronic antigen-induced arthritis. Arthritis & Rheum. 56, 3271-3283

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Regulatory Mechanisms of Lymphocyte Trafficking in Homeostasis and Immunopathogenesis

Regulated lymphocytic recirculation is pivotal in immune system homeostasis and immunopathogenesis. Our work is focused on the role of the chemokine/chemokine receptor system in homeostatic lymphocytic recirculation, systemic and mucosal immune responses, and lymphoid neo-organogenesis during chronic inflammatory or infectious diseases. We further focus on the molecular mechanisms of immune surveillance in preclinical mouse models for B cell lymphoma.

Lymphocytic homeostasis and mucosal immunity

Chemokine receptors regulate peripheral homeostatic lymphocyte recirculation and mucosal immunity

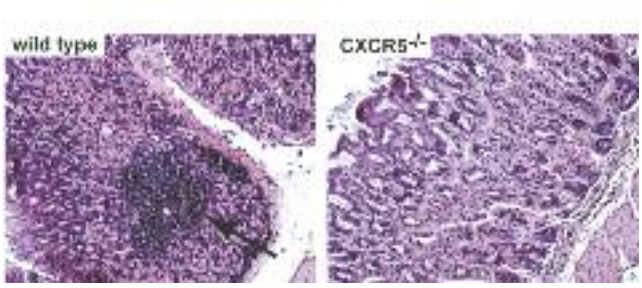
Recently, we showed that the chemokine receptor CCR7 controls not only lymphocyte trafficking to and within secondary lymphoid organs, but also homeostatic migration of T and B lymphocytes through non-lymphoid peripheral tissues. CCR7 deficiency results in massive accumulation of T- and B-lymphocytes in the peritoneal cavity. Mechanistically, an increase in peritoneal lymphocyte numbers is caused by impaired egress of CCR7-deficient lymphocytes from body cavities. Most interestingly, disturbed peripheral recirculation of lymphocytes also resulted in the development of ectopic lymphoid-like follicles and age-dependent histopathological changes in the gastrointestinal tract of CCR7-deficient mice. Since the formation of ectopic follicles precedes the development of epithelial histomorphological changes, we suggest that crosstalk between lymphoid aggregates and their adjacent epithelial tissue might contribute to the development of hypertrophic gastropathy.

The underlying molecular mechanisms have been addressed by performing expression profiling of differentially expressed genes between wild-type epithelial tissue and CCR7$^{-/-}$ epithelial tissue or mucosal lymphoid aggregates. Several transcription factors and signaling molecules were selected on basis of the microarray data and are further characterized as potential mediators involved in this celluar crosstalk.

Chemokine/chemokine receptor function in infectious gastrointestinal cancer models

There is considerable evidence for the involvement of homeostatic chemokines in the formation of tertiary lymphoid tissues during chronic inflammatory processes such as rheumatoid arthritis and *Helicobacter pylori*-induced gastritis. A potential relationship between chronic inflammation, establishment of extranodal tertiary follicles and lymphoma pathogenesis has been inferred from gastric MALT lymphomas in humans. We have analysed the role of the homeostatic chemokine receptor CXCR5 in the formation of mucosal tertiary lymphoid tissue after *H. pylori* infection. CXCR5-deficient mice failed to develop lymphoid aggregates in the glandular stomach and exhibited lower *H. pylori*-specific serum IgG responses compared to infected wild-type mice. Thus, the development of mucosal tertiary ectopic follicles during chronic *H. pylori* infection is strongly dependent on the CXCL13/CXCR5 signaling axis.

To date, we study the function of additional chemokine/chemokine receptors during the pathogenesis of



CXCR5 drives the development of ectopic follicles in the gastric mucosa in H. pylori-infected mice. Histological analysis of paraffin embedded stomachs of CXCR5$^{-/-}$ compared to wild type mice 5-6 month after H. pylori infection showed that ectopic follicles do not develop in CXCR5$^{-/-}$ mice.

MALT-lymphoma and gastric cancer in *in vivo* mouse models.

Immunosurveillance and interactions between tumor cells and its microenvironment

Identification of a chemokine receptor profile characteristic for mediastinal large B-cell lymphoma (MLBCL)

in cooperation with A. Rehm, I. Anagnostopoulos, H. Stein, and B. Dörken, MDC, Charité, Berlin

We have shown that the most frequent lymphoma entities involving the mediastinum can be diagnosed based on the combination of five different homeostatic chemokine receptor stainings. In contrast to diffuse large B cell lymphoma (DLBCLnos) and classical Hodgkin lymphoma, MLBCLs are largely devoid of the homeostatic chemokine receptors CXCR5 and CCR7, and also lack CCR6. These findings suggest that extranodal localization and lack of nodal dissemination in MLBCL are related to the expression of those lymph node addressins. Conversely, expression of these receptors on essentially all B cell neoplasms with a widespread nodal dissemination provide a rationale for the therapeutic targeting of homeostatic chemokine receptors with antibodies or antagonistic compounds.

Cellular interactions between lymphoma cells and their accessory or tumor stroma cells

in cooperation with A. Rehm, and B. Dörken, MDC, Charité, Berlin

Based on our phenotypical analysis of chemokine/chemokine receptor expression in MLBCL, we now focus on the cellular interactions between lymphoma cells and their accessory or tumor stroma cells. Investigations on the relationship between lymphoma cells and its local immune environment includes the analysis of active immune escape mechanisms in murine B cell lymphoma. We backcrossed the transgenic malignant lymphoma mouse strain, *Eμ-Myc*, onto diverse chemokine receptor KO strains, which will help to decipher the influence of tumor cell homing within a specific lymph node and splenic niche onto disease onset and progression. A more profound understanding of mechanisms that may cause non-Hodgkin lymphoma to be addicted to the local microenvironment could provide new targets for therapeutic intervention.

Immunoregulatory functions of the tumor-associated antigen EBAG9/RCAS1

in cooperation with A. Rehm, and B. Dörken, MDC, Charité, Berlin

This project studies whether the estrogen-inducible tumor-associated antigen, EBAG9, has a concurrent impact on T cell-mediated tumor immunosurveillance. Cytotoxic T lymphocytes (CTL) are essential for immunosurveillance and score cells for the display of tumor-derived peptides. For target cell destruction, CTLs employ polarized secretion of lytic granules. We generated EBAG9 knockout mice and characterized the consequences of its deletion in CTL-mediated immune responses. Loss of EBAG9 amplifies the release of lytic granules and confers CTLs with an enhanced cytolytic activity, in all likelihood through improved formation of fusion- and release-competent secretory lysososomes.

With regard to tumor immunosurveillance and tumor immunotherapy, modulating the cell biological roadblocks in T cell activation and cytolytic capacity on a single cell level emerges as a strategy to increase avidity and to strengthen anti tumor T cell efficiency. Our identification of the estrogen tunable repressor of cytotoxic T cell activity, EBAG9, will allow us to suppress its activity through pharmacological estrogen receptor blockade.

Selected Publications

Höpken, UE, Winter, S, Achtman, AH, Krüger, K, Lipp, M. (2010). CCR7 regulates lymphocyte egress and recirculation through body cavities. J. Leukoc. Biol. *In press.*

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Rüder, C, Höpken, UE*, Wolf, J, Mittrücker, H-W, Engels, B, Erdmann, B, Wollenzin, S, Uckert, W, Dörken, B, Rehm, A*. (2009). The tumor-associated antigen EBAG9 negatively regulates the cytolytic capacity of mouse CD8+ T cell. J. Clin. Invest. 119: 2184-2203*shared corresponding author

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Molecular Immunology and Gene Therapy

Most of the current experimental cancer models do not reflect the pathophysiology of real-life cancer. Cancer usually occurs sporadically and is clonal in origin. Between tumor initiation and progression clinically unapparent pre-malignant cells may persist for years or decades in humans. More recently, mouse models of sporadic cancer have been developed. The mouse germ-line can be engineered with high precision so that defined genes can be switched on and off in the adult organism, ideally in a locally and timely controlled fashion. However, analysis of the immune response against sporadic tumors requires the knowledge of a tumor antigen.

In the last two years our group focused on several areas in cancer immunology. We analysed at which point of cancer development T cells become aware of the cancer cells. We asked whether the initial recognition of cancer cells by cytotoxic T-lymphocytes (CTL) results in tumor immunity or tolerance. Additionally, we employed different strategies of cellular therapy.

Immunogenicity of premalignant lesions is the primary cause of general cytotoxic T lymphocyte unresponsiveness

Cancer is sporadic in nature, characterized by an initial clonal oncogenic event and usually a long latency. When and how it subverts the immune system is unknown. We show, in a model of sporadic immunogenic cancer, that tumor-specific tolerance closely coincides with the first tumor antigen recognition by B cells. During the subsequent latency period until tumors progress, the mice acquire general cytotoxic T lymphocyte (CTL) unresponsiveness, which is associated with high transforming growth factor (TGF)β 1 levels and expansion of immature myeloid cells (iMCs). In mice with large nonimmunogenic tumors, iMCs expand but TGF-β 1 serum levels are normal, and unrelated CTL responses are undiminished. We conclude that (a) tolerance to the tumor antigen occurs at the premalignant stage, (b) tumor latency is unlikely caused by CTL control, and (c) a persistent immunogenic tumor antigen causes general CTL unresponsiveness but tumor burden and iMCs per se do not.

Engineering antigen-specific primary human NK cells against HER-2 positive carcinomas

NK cells are promising effectors for tumor adoptive immunotherapy, particularly when considering the targeting of MHC class I low or negative tumors. Yet, NK cells cannot respond to many tumors, which is particularly the case for nonhematopoietic tumors such as carcinomas or melanoma even when these cells lose MHC class I surface expression. Therefore, we targeted primary human NK cells by gene transfer of an activating chimeric receptor specific for HER-2, which is frequently overexpressed on carcinomas. We found that these targeted NK cells were specifically activated upon recognition of all evaluated HER-2 positive tumor cells, including autologous targets, as indicated by high levels of cytokine secretion as well as degranulation. The magnitude of this specific response correlated with the level

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of HER-2 expression on the tumor cells. Finally, these receptor transduced NK cells, but not their mock transduced counterpart, efficiently eradicated tumor cells in RAG2 knockout mice as visualized by in vivo imaging. Taken together, these results indicate that the expression of this activating receptor overrides inhibitory signals in primary human NK cells and directs them specifically toward HER-2 expressing tumor cells both in vitro and in vivo.

A safeguard eliminates T cell receptor gene-modified autoreactive T cells after adoptive transfer

By transfer of T cell receptor (TCR) genes, antigen specificity of T cells can be redirected to target any antigen. Adoptive transfer of TCR-redirected T cells into patients has shown promising results. However, this immunotherapy bears the risk of autoreactive side effects if the TCR recognizes antigens on self-tissue. Here, we introduce a safeguard based on a TCR-intrinsic depletion mechanism to eliminate autoreactive TCR-redirected T cells in vivo. By the introduction of a 10-aa tag of the human c-myc protein into murine (OT-I, P14) and human (gp100) TCR sequences, we were able to deplete T cells that were transduced with these myc-tagged TCRs with a tag-specific antibody in vitro. T cells transduced with the modified TCR maintained equal properties compared with cells transduced with the wild-type receptor concerning antigen binding and effector function. More importantly, therapeutic in vivo depletion of adoptively transferred T cells rescued mice showing severe signs of autoimmune insulitis from lethal diabetes. This safeguard allows termination of adoptive therapy in case of severe side effects.

Selected Publications

Kieback, E., Charo, J., Sommermeyer, D., Blankenstein, Th. And Uckert, W. (2008). A safeguard eliminates T cell receptor gene-modified autoreactive T cells after adoptive transfer. Proc. Natl. Acad. Sci. USA 105: 623-628.

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Kammertoens, T. and Blankenstein, T. (2009) Making and circumventing tolerance to cancer. Eur. J. Immunology, in press.

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Molecular Cell Biology and Gene Therapy

The introduction of T cell receptor (TCR) genes into T cells has been developed over the past years as a strategy to induce defined antigen-specific T cell immunity. The potential value of TCR gene therapy was well established in mouse models and the feasibility of infusion of TCR gene-modified autologous T cells was shown in first clinical studies. The next steps will be the translation of TCR gene therapy from an experimental technique into a robust and safe clinical strategy.

The focus of our group lies on the therapy of cancer and viral diseases using TCR gene therapy. We address questions related to: generation of T cells with new antigen specificity, modification of TCR genes to generate T cells with high functional avidity, safety aspects of TCR gene-modified T cells with respect to the recognition of self-antigens, adoptive transfer of TCR gene-modified T cells in mice as preclinical models, and optimization of TCR transfer vectors.

Redirection of T cell antigen specificity by TCR gene transfer

Matthias Leisegang, Daniel Sommermeyer, Lilian Stärck, Peter Meyerhuber, Haike Gelfort in collaboration with Helga Bernhard, Antonio Pezzutto, Dolores Schendel

We have molecularly cloned TCRα/β genes recognizing tumor-associated antigens (HER2, Mart-1, Melan-A, NY-ESO-1, RCC-26, RCC-53, Survivin, Tyrosinase), virus-specific antigens (CMV, EBV-EBNA3a, EBV-LMP2a), and model antigens (LCMV-gp33, ovalbumin). Using retrovirus vectors, TCR genes were transferred into T cell lines and primary T cells of mouse and human origin. TCR-α and β-chains were expressed on the cell surface and functionality of transgenic TCRs was demonstrated by antigen recognition, cytokine secretion and lysis of tumor cells.

Designer T cells by TCR optimization

Daniel Sommermeyer

To increase the avidity of TCR-redirected T cells, we introduced different modifications into the TCR genes (codon optimization, additional disulfide bond, murinization of constant regions of human TCR chains) and demonstrated exemplarily for the NY-ESO-1 TCR that functional T cell avidity can be considerably improved (Figure).

One of these modifications – "murinization" – which replaces the human TCR and TCR constant regions by their murine counterparts was analyzed in detail. Using a series of mouse-human hybrid constructs, we identified nine amino acids responsible for the improved expression of murinized TCRs. Five critical amino acid exchanges were identified in the TCR constant region, with exchange of an acidic glutamic acid (human) for a basic lysine (mouse) at position 18 of the constant region, being most important. For the TCR constant region, a domain of four amino acids was sufficient for improved expression. The minimal murinized TCR variants enhanced expression of human TCRs by supporting preferential pairing of transferred TCR chains. Furthermore, usage of minimal murinized TCR chains improved the function of transduced primary human T cells when compared to cells transduced with wild-type TCRs. For TCR gene therapy, the utilization of minimal

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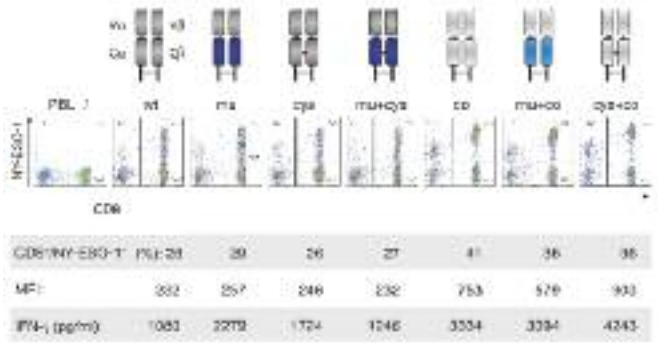

Figure *Modifications increase the avidity of TCR gene-modified T cells. A NY-ESO-1-reactive wild-type (wt) TCR was modified by murinization (mu; substitution of human TCR constant regions by mouse counterparts), introduction of a cysteine bond (cys), codon optimization (co) or combinations of modifications. Transduction efficiency (%), TCR expression level (MFI) and fuctionality of TCR gene-modified human PBL after co-culture with antigen presenting tumor cells (IFN-γ release) were determined.*

instead of completely murinized constant regions dramatically reduces the number of foreign residues and thereby the risk for immunogenicity of therapeutic TCRs.

Designer T cells by vector optimization

Matthias Leisegang, Peter Meyerhuber, Elisa Kieback, Daniel Sommermeyer, Simone Reuß in collaboration with Hans Stauss,

For clinical application of TCR-redirected T cells, efficient functional expression of the transgenic TCR is a key prerequisite. We compared the influence of the transgene cassette on the expression and function of the murine TCR P14 (recognizing a LCMV gp33 epitope) and the human TCR WT-1 (recognizing an epitope of the tumor-associated antigen WT-1). We constructed different vectors, in which TCR-α and β-chain genes were (i) linked by an IRES element, (ii) combined by a 2A peptide or (iii) introduced into two individual retroviral constructs. We found that IRES-, but not 2A-employing TCR expression is hampered in primary T cells, where the transgenic TCR has to compete with endogenous TCR chains. Differences in expression were independent of copy number integration as shown by quantitative PCR. Thus, linking TCR-α and β-chain genes by a 2A peptide seems superior to an IRES element and to single gene vectors for TCR expression and T cell function.

Construction of TCR-retrovirus packaging cell lines

Simone Reuß, Matthias Leisegang

We constructed suspension cell line-based packaging cells derived from human lymphoblastoid -Jurkat cells to produce TCR-retroviruses. These cells lack endogenous TCRβ-chains and are unable to present CD3 molecules on the cell surface. Packaging functions were transferred into Δβ-Jurkat cells by electroporation of plasmids encoding the gag-pol gene of murine leukemia virus (MLV) and the GALV or MLV-10A1 env gene. Cell clones expressing high amounts of gag-pol and env gene products were determined by RT-PCR and Western blot analysis. Upon introduction of a TCR-encoding retroviral vector, Δβ-Jurkat packaging cells shifted through the expression of the transgenic TCR-chain from CD3-negative to CD3-positive cells. CD3 high-expressing packaging cells were enriched by FACS sorting and produced high-titer TCR-retrovirus containing supernatant.

Selected Publications

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Uckert, W, Schumacher, T. (2009). TCR transgenes and transgene cassettes for TCR gene therapy: status in 2008. Cancer Immunol. Immunother. 58: 809-822.

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Biology and Targeted Therapy of Lymphoma

Studying the molecular basis underlying lymphoid cell development and differentiation is one of the key approaches to understand the pathways leading to disease. We are interested in the mechanisms that give rise to the development of human hematologic malignancies, in particular to that of classical Hodgkin lymphoma (cHL) and anaplastic large cell lymphoma (ALCL). Our work focuses on the characterization of the molecular basis for the dedifferentiation program of these lymphoid cell-derived malignancies and furthermore aims to characterize molecular defects that are responsible for tumor cell transformation, proliferation and apoptosis protection. It is the ultimate aim of our work to identify targets for the development of new treatment strategies.

Characterization of deregulated transcription factor networks in Hodgkin lymphoma and anaplastic large cell lymphoma

S. Mathas, M. Janz, B. Dörken in cooperation with H. Stein (Charité)

Using classical Hodgkin lymphoma (cHL) as a model system, we are investigating the role of transcription factors in lymphoma development. Malignant transformation of hematopoietic cells is associated with profound alterations in the transcriptional program resulting in deregulated proliferation, differentiation, and apoptosis. It is our aim to identify genes and transcription factor networks specifically deregulated in malignant lymphoma cells. Our work revealed in HRS cells a functional disruption of the B cell-specific transcription factor network, which is composed of the transcription factors E2A, EBF, and Pax5. In particular, the B cell-determining transcription factor E2A is inhibited by its overexpressed antagonists activated B cell factor 1 (ABF-1) and inhibitor of differentiation 2 (Id2). Importantly, these factors are able to down-regulate the expression of B cell-specific genes and to allow up-regulation of B lineage-inappropriate genes. We could also show that B lineage-inappropriate genes like IL-21 contribute to the attraction of immune cells supporting the growth of HRS cells. In addition, the loss of the B cell phenotype in primary effusion lymphoma (PEL) is based on similar molecular mechanisms, and in these cells reconstitution of B cell specific E2A activity resulted in induction of apoptosis. These data support the concept that the loss of lineage-specific transcription factors in lymphoid cells might be linked to the process of malignant transformation. Thus, further understanding of the dedifferentiation process in lymphoid cells provides a basis for the development of novel targeted therapeutics for lymphoma therapy.

Identification of survival pathways of lymphoma cells for the development of new therapeutic strategies

M. Janz, S. Mathas, B. Dörken

In continuation of earlier work of our group, we focus on the analysis of the NF-κB and AP-1 transcription factor system with respect to apoptosis resistance and proliferation. These pathways are investigated not only in HRS of cHL, but also in lymphoma entities such as anaplastic large cell lymphoma (ALCL) and multiple myeloma. Recent results show that overexpression of the NF-κB/IκB family member Bcl-3 constitutes a novel molecular defect of the NF-κB system in cHL and ALCL.

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Bcl-3 might be involved in apoptosis protection of these cells. Furthermore, we have shown that AP-1 is involved in the dedifferentiation process of HRS cells by maintaining high expression of the E2A antagonist inhibitor of differentiation 2 (Id2), and the overexpression of the AP-1 family member Fra2 in ALCL cells contributes to their malignant transformation. The AP-1 activity might further be enhanced by a specific overexpression of the CREB family member ATF3. ATF3 is specifically overexpressed in HRS and ALCL tumor cells and protects at least HRS cells from apoptosis. In addition, we showed that p53-dependent apoptosis can be induced in HRS cells by the MDM2-antagonist nutlin-3, and thus the activation of the p53 pathway might represent a novel treatment strategy for cHL. These data provide insights into the deregulated apoptosis and survival signaling pathways in HRS cells.

Analysis of the mechanisms leading to formation of the ALCL-specific translocation t(2;5)(p23;q35)

S. Mathas, B. Dörken in cooperation with T. Misteli (NCI, Bethesda)

Whereas the identification and characterization of translocations rapidly increases, little is known about the mechanisms how translocations occur *in vivo*. Using anaplastic large cell lymphoma (ALCL) with and without the characteristic non-random translocation t(2;5)(p23;q35) as model system, we study the mechanisms of formation of t(2;5)(p23;q35) translocations in these cells. We could show that several genes surrounding the ALCL translocation breakpoint are deregulated regardless of whether the tumor contains the t(2;5). The affected genes include the oncogenic transcription factor Fra2 (located on 2p23), the HLH protein Id2 (2p25) and the oncogenic tyrosine kinase CSF1-receptor (5q33.1), and their up-regulation promotes cell survival and repression of T cell-specific gene expression programs, a feature that is characteristic for ALCL. By 3D interphase FISH analyses we demonstrated that the deregulated genes are in spatial proximity within the nuclear space of t(2;5)-negative ALCL cells, facilitating their translocation. These data suggest that deregulation of breakpoint proximal genes occurs prior to formation of translocation and that aberrant transcriptional activity of genome regions is linked to their propensity to undergo chromosomal translocations.

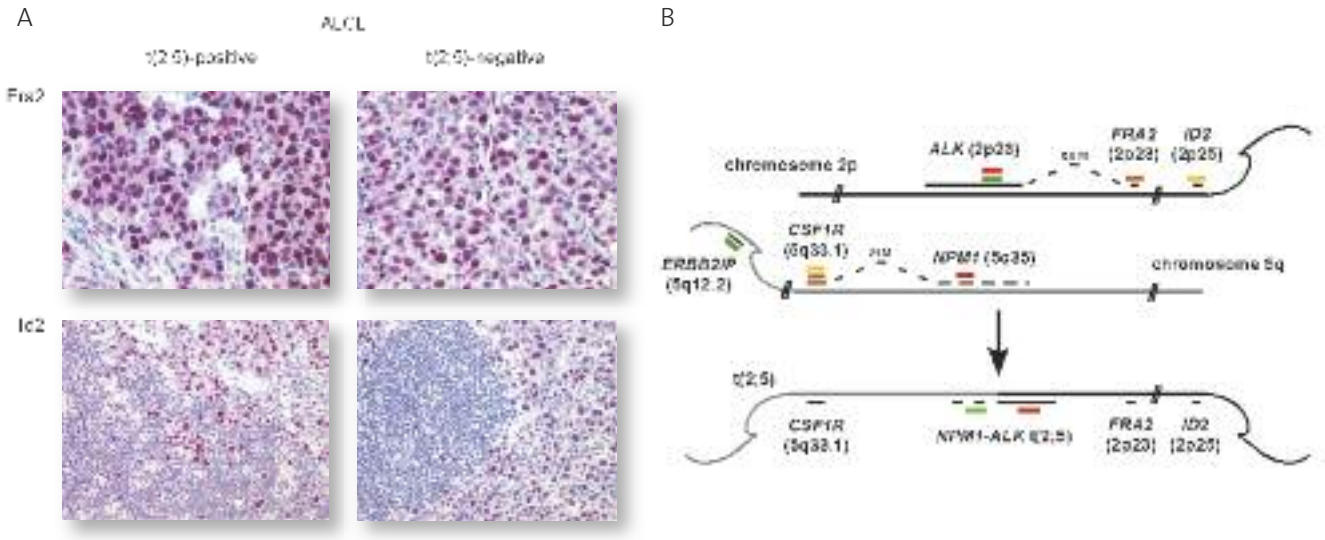
How to deliver cytotoxic agents during effector T cell mediated adaptive immune responses

A. Rehm, B. Dörken in cooperation with U.E. Höpken, T. Willnow, MDC

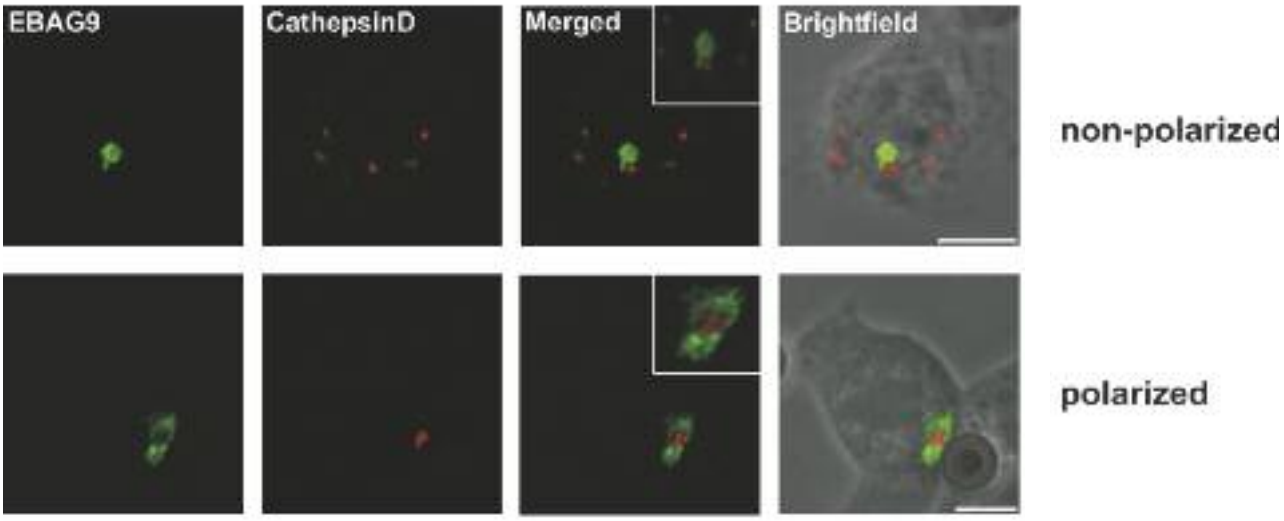
Estrogen plays a critical role in the regulation of growth, development and cell type-specific gene expression in various tissues, including the immune system. Alterations in the response to estrogen are associated with hormone-dependent diseases, such as breast and ovarian cancer. Adjuvant therapies with estrogen-receptor inhibitors mounted to a tremendous improvement of tumor-free survival among patients, which was explained primarily through the inhibition of the pro-proliferative effect of estrogen in tumor cells itself. However, implications for a concurrent impact on the immune system remained unclear. We have characterized an estrogen-inducible tumor-associated antigen, EBAG9, through engineered deletion of the gene in mice.

In cytotoxic T lymphocytes from these mice, equipped with lytic granules that undergo polarized transport and exocytosis in a Ca^{2+}-dependent manner, release of cytolytic mediators was enhanced, an effect that translated into the rapid clearance of intracellular bacteria. Furthermore, a tumor-peptide labeled target cell population was eliminated in an accelerated fashion. Thus, under physiological conditions EBAG9 tempers lymphocyte killing activity. Upon estrogen-induced enhanced gene expression, EBAG9 could act as an inhibitor of full effector T cell activation with an ensuing lack of immunosurveillance.

While the function of the neuronal sorting receptors SorLA/LR11 and Sortilin within the neuronal system has been well established, little is known about their role in the immune system. In a Sortilin-genetically deleted mouse strain we have elucidated that this sorting receptor plays a pivotal and non-redundant role during the cytotoxic effector function. However, unlike EBAG9-



Gene deregulation and its impact on formation of translocations in anaplastic large cell lymphoma (ALCL). *(A) Immunohistchemistry of Fra2 and Id2 in ALCL carrying (left) or lacking (right) t(2;5). Cells with positive signals are stained in red. Large neoplastic cells show a strong nuclear staining for Fra2 and Id2, whereas no (Fra2) or only weak (Id2) signals are detectable in the small non-neoplastic cells. (B) Schematic view of the breakpoint proximal genes up-regulated in ALCL cells showing the localization of* FRA2 *(located on 2p23),* ID2 *(2p25), and* CSF1R *(5q33.1). In the case of t(2;5), the fusion of* NPM *(5q35) and* ALK *(2p23) results in the* NPM-ALK *fusion gene. Colored bars indicate positions of probes used for FISH in Mathas et al., PNAS, 2009.*



Activation-dependent intracellular redistribution of EBAG9 toward the immunological synapse

EBAG9 (green) relocalizes toward the immunological synapse when cytotoxic T lymphocytes are conjugated with CD3/CD28 coated microbeads, which act as surrogate antigen-presenting cells (opaque structures).

CTLs were mixed with CD3/CD28 coated microbeads, incubated for 5 min at 37°C, plated on coverslips, and incubated for another 30 min before fixation and permeabilization. Lytic granules were stained with anti-Cathepsin D antibody (red). A non-conjugated and non-polarized T cell is shown in the top row. Polarized T cells with conjugated beads are shown below. Images were obtained with a Zeiss LSM 510 confocal microscope setup. Scale bars, 5 micron. Insets have additional magnifications of 2- to 3-fold.

deficient mice, both major effector T cell populations, CD8+ cytotoxic T cells and CD4+ Th1 cells, were found to be affected in Sortilin KO animals.

From both animal models we infer that the modulation of cell biological roadblocks in T cell activation and cytolytic capacity on a single cell level emerges as a strategy to increase avidity and strengthen anti tumor T cell efficiency. Conversely, augmentation of EBAG9 or Sortilin activity could turn out to be beneficial in the treatment of autoimmune disorders.

Selected publications

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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. We have recently concluded a pilot clinical study with a gene-modified tumor cell vaccine for treatment of advanced metastatic renal cancer. A follow-up study by combining this vaccine with antibody therapy to modulate regulatory T cells (T-regs) is in preparation in patients who responded to therapy with thyrosine kinase inhibitors such as sorafenib and sunitinib.

Clinical vaccination studies using dendritic cells or modified tumor cells

Jörg Westermann and J. Kopp, in cooperation with Th. Blankenstein, W. Uckert and D. Schendel (GSF, Munich).

In renal carcinoma (RCC), a gene-modified, 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. Based on our recent data concerning the numbers of regulatory T cells in RCC patients undergoing therapy with the multikinase inhibitors sorafenib and sunitinib we are planning a follow-up trial in conjunction with antibodies that increase T-cell stimulation by reducing regulatory T-cells.

In a recently concluded clinical vaccination trial using *in vitro*-generated dendritic cells (DC) in patients with chronic myeloid leukemia (CML) we have seen reduction in the tumor load in 50% of the patients. A follow-up multicenter national trial is starting in fall 2009 in CML patients with clinical but not molecular remission upon treatment with the tyrosine-kinase inhibitor imatinib.

DNA Vaccination: preclinical models

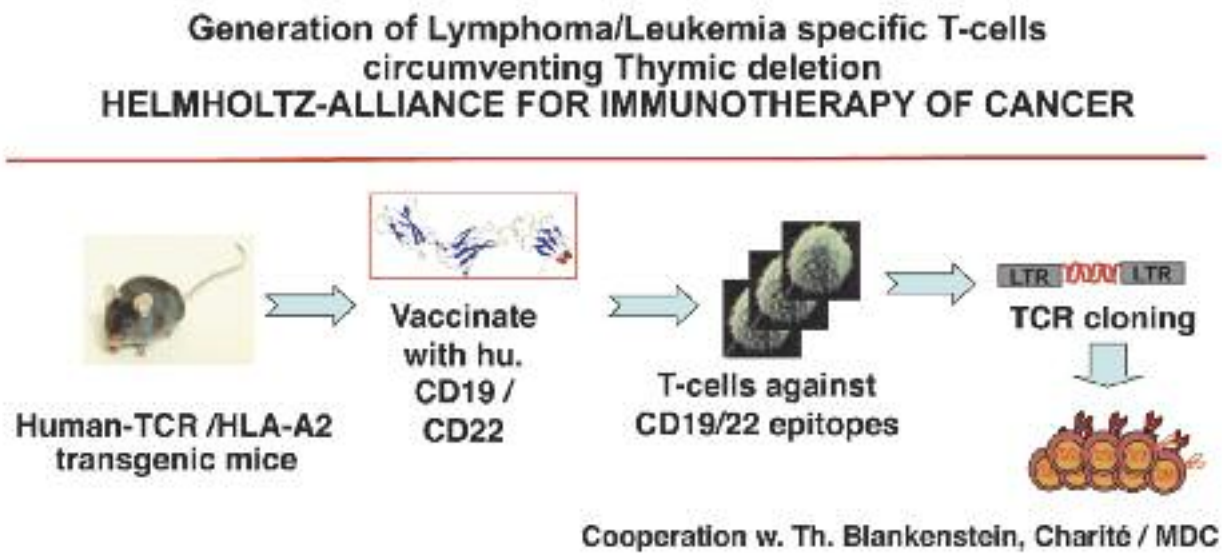
Jörg Westermann, Tam Nguyeng Hoay and Sonia Waiczies in cooperation with U. Höpken and M. Lipp and Physikalische Bundesanstalt, Berlin

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). In cooperation with the group of M. Lipp (Molecular Tumor Genetics) we have explored the possibility of recruiting immune cells at the vaccine site by inserting DNA sequences coding for the chemokines CCL19 and CCL21 as possible adjuvants. Coexpression of CCL19 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 novel gene-gun for vaccine application (Cooperation with the Robert-Koch Institute). We have set up a preclinical model using the human breast-cancer associated antigen Her-2 as a target antigen to explore the potential use of a vaccine for breast cancer patients. S. Vaiczies is exploring magnetic resonance imaging to study dendritic cell migration in vivo.

Immunity against EpCam (Epithelial Cell adhesion molecule) / CD3 kappa as an immunomodulatory polypeptide

Oliver Schmetzer in cooperation with P. Schlag and G. Moldenhauer (DKFZ)

Heteroclitic peptides derived from the Epithelial Adhesion Molecule EpCam appear able to induce a strong T-cell stimulation by upregulating the TCR. A splice vari-



ant of CD3 delta coding for a 45-mer polypeptide appears to mediate the increase of the T-cell receptor density. Characterization of this polypeptide and evaluation of its properties as an immunomodulatory agent is ongoing.

Adoptive T-cell therapy for lymphomas

Tuan Duc Nguyen and Nina Schmolka in cooperation with T. Blankenstein and W. Uckert

Hodgkin, Nasopharyngeal Carcinomas, and Post-Transplant Lymphoproliferative Disorders (PTLD) are associated with Epstein-Barr Virus (EBV) infection. CD8 and CD4 clones have been generated against EBV proteins. The corresponding TCRs have been cloned in expression vectors for generation of transgenic T cells for adoptive therapy of EBV-associated diseases. Optimization of TCR expression led to improved killing efficiency of gene-modified T cells in vitro.

Moreover, the B-cell antigens CD19 and CD22 are appealing targets for a transgenic T cell recognition. Generation of TCR vectors recognizing epitopes of these antigens is being pursued in cooperation with Th. Blankenstein in a murine system with humanized TCR gene sequences.

Development of less toxic immunotoxins targeting CD22 positive lymphomas

Tuan Duc Nguyen, Oliver Schmetzer in cooperation with G. Moldenhauer (DKFZ)

Immunoconjugates coupled to toxic agents have potent biological activity but side effect that can severely limit their use. In a new approach, our group generates immunotoxins which are based on non-toxic substances like dithiocarbamate-based compounds or catechins which has been isolated from green tea. Coupled to an Anti-CD22-antibody, which is rapidly internalized from lymphoma cells, leads to apoptosis induction in cancer cells.

Selected Publications

Schmetzer, O, Moldenhauer, G, Riesenberg, R, Pires, JR, Schlag, P, Pezzutto, A. (2005). Quality of recombinant protein determines the amount of autoreactivity detected against the tumor-associated epithelial cell adhesion molecule antigen: low frequency of antibodies against the natural protein. J Immunol. 174, 942-952.

Sebelin-Wulf, K, Nguyen, TD, Oertel, S, Papp-Vary, M, Trappe, RU, Schulzki, A, Pezzutto, A, Riess, H, Subklewe, M. (2007). Quantitative analysis of EBV-specific CD4/CD8 T cell numbers, absolute CD4/CD8 T cell numbers and EBV load in solid organ transplant recipients with PLTD. Transpl Immunol. 17, 203-210.

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Patent applications

EpCAM MHC-Klasse-II bindende Peptide und davon abgeleitete Mutanten als Verstärker der zellulären tumorreaktiven immunantwort (MDC 0506 EP)

Peptides regulating the surface expression of the T cell receptor

European Patent Application EP1870420

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Clinical and Molecular Oncology

Virtually all medical anticancer therapies rely on the induction of cell cycle arrest or cell death in the malignant cells. Consequently, the analysis of such genetic events allows for the identification of patients at risk for an insufficient response to treatment and poor survival. Such analyses therefore provide a rational basis for a molecular understanding of the response to anticancer therapies and the clinical use of cancer therapeutics. The aim of the group is, therefore, to define genetic defects in cancer that result in aggressive disease, poor prognosis, and resistance to clinical cancer therapy. To this end, we have established an extensive genotyping and functional genomics program in solid tumors and leukemias. Recent data indicate that specific defects in cellualr stress pathways leading to cellular resistance may be overcome by rationally selected targeted anticancer drugs. In addition, these systems are exploited to gain insights into novel aspects of cell cycle and cell death regulation and their intricate interactions.

Understanding resistance to anticancer therapy

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 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. We have investigated the consequences of genetic defects in genes acting as effectors or inducers of p53 that trigger apoptosis and cell cycle arrest programs upon genotoxic stress. In this context, we recently described selective loss of multiple BH3-only proteins, pro-apoptotic homologs of the Bcl-2 family, including Nbk and Bim in renal carcinoma. 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.

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 antiapoptotic and proapoptotic 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 heterocomplex formation as well as for their cell death inducing capacity. Structural and functional analyses revealed that the proapoptotic homologs can be subdivided into the Bax subfamily and the growing BH3-only subfamily. BH3-only proteins link upstream signals from different cellular or functional compartments to the mitochondrial apoptosis pathway (see figure). 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 Bax (but not Bak) indirectly, through a ER-initiated death pathway that has been recently elucidated by our group.

The aim of our work is to gain structural and functional insights into how these subfamilies promote or inhibit cell death signals and how these properties may be utilized for development of apoptosis-promoting

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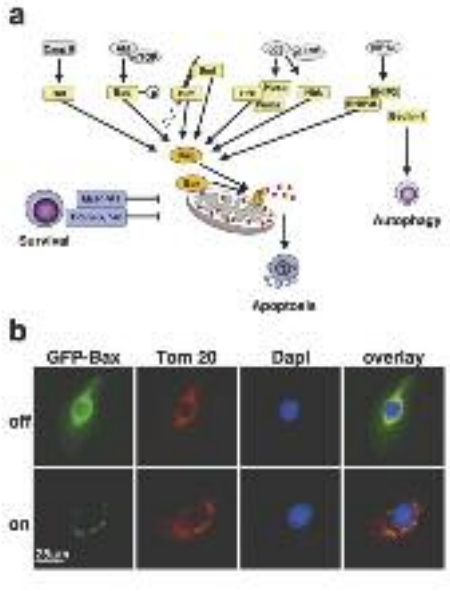
cancer therapies. Our studies therefore deal with questions such as how cell cycle stress responses including anticancer therapies and oncogene deregulation feed into the mitochondrial death pathway. We recently established that Nbk stabilizes the anti-apoptotic multidomain protein Mcl-1 that acts as an endogenous inhibitor of Bak. This fully explains the entirely Bax dependent induction of apoptosis by Nbk. Ongoing work addresses the transcriptional control of Nbk expression and its functional involvement in the regulation of cell death following ER stress responses. Regulation of the Bak pathway by Mcl-1 (but not Bcl-x_L) is of general relevance for targeted cancer therapy and was shown to mediate TRAIL resistance in Bax deficient carcinoma cells.

Cell cycle and apoptosis

Using the apoptosis, cell cycle arrest and senescence inducing tumor suppressor gene $p14^{ARF}$ expression as a model system, we explore the intricate interconnections between cell cycle stress responses and apoptosis induction. $P14^{ARF}$ expression is induced upon cellular stress, especially following deregulation of oncogenes. While physical interaction of $p14^{ARF}$ with numerous regulatory proteins, induction of p53-dependent cell cycle phenomena and cellular senescence by $p14^{ARF}$ are well established, little is known how $p14^{ARF}$ induces cell death. Notably, we established that the induction of mitochondrial apoptosis by $p14^{ARF}$ is entirely independent from p53 and Bax in p53-deficient cells where Bak can fully complement for Bax function. Apoptosis is mediated, at least in p53-proficient cells, via the BH3-only protein Puma/bbc3 and relies on procaspase-3 for cell death execution. In contrast to apoptosis induction, the triggering of a G1 cell cycle arrest (and presumably premature cellular senescence) by $p14^{ARF}$ is entirely dependent on p53 and $p21^{CIP/WAF-1}$, indicating that the signaling pathways for $p14^{ARF}$-induced G1 arrest and apoptosis induction dissociate upstream of p53. Noteworthy, loss of p21 and/or 14-3-3σ strongly enhances apoptosis induction by $p14^{ARF}$. Nonetheless, we recently demonstrated that, in the absence of functional p53 and/or p21, $p14^{ARF}$ triggers a G2 cell cycle arrest by downregulation of cdc2-kinase activity, protein expression, and cytoplasmic localization in these cells whereas $p14^{ARF}$is localized to the nucleus, i.e. mediates cdc2 sequestration and induction of mitochondrial apoptosis through an indirect mechanism. Such p53-independent mechanisms of $p14^{ARF}$ induced apoptosis and arrest in the cell division cycle represent fail-safe mechanisms that allow for efficient growth suppression following induction of $p14^{ARF}$-mediated stress responses in p53 pathway deficient cells.



Function of BH3-only proteins as death sensors. *A: BH3-only proteins act as functional interface between death signals and the mitochondrial apoptosis pathway. Anti-apoptotic Bcl-2 proteins put an at least dual layer of protection on activation of Bax/Bak that redistribute upon activation to form pores in the outer mitochondrial membrane for the release of pro-apoptotic factors such as cytochrome c. B: Conditional adenoviral expression of Nbk induces redistribution of Bax (EGFP, green) to mitochondria (TOM20, red) and a punctuate formation of Bax clusters due to oligomerization. Blue colour: DAPI stained nuclei. Mitochondria fragment and cluster around the nucleus in apoptotic cells (on condition) as compared to the control (off).*

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Cellular Immunology of Autoimmune reactions – Controlling the Balance between Effector and Suppressor T cells

Direction and strength of the immune response is largely controlled by the equilibrium between effector and suppressor T cells. While effector T cells drive proinflammatory reactions to eradicate pathogens and transformed cells, suppressor T cells prevent autoimmune reactions as well as the collateral damage by keeping the effector cells in check. It is now generally accepted that regulatory T cells (Treg) are a key suppressor population responsible for the maintenance of peripheral tolerance. Characteristic marker of Treg cells is the transcription factor Foxp3. Specific aim of the group is to understand the composition and function of Treg subsets and to explore their role for future immune interventions in autoimmune diseases and cancer.

Antigen-specific Recruitment of Treg cells and Treg-based cell therapies

Cooperation with the SFB650, Berlin, the BMBF network project 'BIOTIA', and the 'Singapore Immunology Network' (SIgN), Singapore

Treg cells may offer a novel perspective for the treatment of autoimmune diseases as the antigen-specific recruitment of these cells could result in dominant protection from the autoimmune attacks. In several experimental animal models we have demonstrated that repeat antigens consisting of linear copies of a T cell epitope are particularly effective to inducing this effect. This applies for the treatment of multiple sclerosis-like autoimmune disease (EAE), as well as for experimental autoimmune neuritis (EAN) and an animal model of type I diabetes (T1D). Recent studies further suggest that the strategy is also widely used by parasites such as *plasmodium spec* to mediate immune evasion. In a "bionics approach" tolerogenic repeat regions identified in the malaria parasite *plasmodium falciparum* are currently being tested in the autoimmune model of multiple sclerosis.in the context of the BIOTIA network.

In another approach the group has developed a method that provides access to 'untouched' human Treg cells. It has been documented numerous times in mice that the adoptive transfer of Treg cells is a very effective cell-therapeutic approach to treat inflammatory diseases and syndromes. Translation of these approaches into clinical praxis however was hindered by the lack of suitable cell isolation techniques. The new approach allows now obtaining highly pure populations of human Treg cells particularly suitable for therapeutic applications In a mouse model of 'graft vs. host disease' (GvHD), the cells have been proven already to be capable of controlling the disease. In a joint project with SIgN, a clinical phase I trial has been launched in Singapore, in which 'untouched' Treg cells will be used to treat GvHD in leukaemia patients.

Extracellular ATP and CD39+ Treg cells

Cooperation with Santa Lucia Foundation, Rome, Italy, the GRK 1258, Berlin and SIgN, Singapore

As indicator of 'non-natural' (necrotic) cell death ATP is released through the damaged cell membrane into the extracellular space. It triggers various proinflammatory reactions such as the maturation of dendritic cells (DC) and the regulation the inflammasome-mediated release IL-1. The latter is also known as 'endogenous

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pyrogen' as it acts as key-factor in igniting the immune response. We have recently shown that Treg cells express with CD39 a surface ATPase, able to degrade extracellular ATP to ADP and AMP. Studies in the animal model of multiple sclerosis (MS) as well as in human MS indicate that CD39+ T_{REM} cells play indeed a major role in controlling the disease. CD39-/- mice are susceptible to EAE and MS patients have strikingly reduced numbers of CD39+ Treg cells in the blood (Fig. 1). Thus the control of extracellular ATP-levels by these cells seems tom be crucial in suppressing the autoimmune disease.

Notably, the individual variations in the cell number seem to have genetic causes. Recent studies in Singapore indicate that the CD39-Treg phenotype is much more frequent in the Asian population. Given its apparent importance of this polymorphism for inflammatory immune responses a study has been launched in Singapore to determine whether a 'single nucleotide polymorphism' (SNP) can be identified that and can be used as a diagnostic indicator explains the underlying mechanism.

The impact of 'MHC-loading enhancer' (MLE) on the immune response

Cooperation with the BMBF network project 'MHCenhancer' and the European MC-RTN 'Drugs for Therapy'

Class II MHC molecules are receptor molecule presenting antigens in the form of short peptides for the surveillance by CD4+ T cells. Cell surface MHC molecules that have lost their ligand rapidly inactivate. They acquire a 'non-receptive state', presumably to prevent the 'accidental' exchange of peptide ligands on the cell surface. While this safe guard mechanism minimizes the uncontrolled loading of MHC molecules, it also inhibits the effective antigen-loading needed for peptide vaccinations. During the past years the group had identified a number of small molecular compounds termed 'MHC-loading enhancer' (MLE) that can bypass this safety mechanism. By acting directly on cell surface MHC molecules they can reconstitute the non-receptive MHC molecule in a catalytic fashion.

In cooperation with partners from the 'Leibnitz Institute of Molecular Pharmacology' (FMP) and other members of the 'MHCenhancer' network MLE were found to target a defined pocket of the class II MHC molecule. The transient occupation of this pocket stabilizes the peptide-receptive state in a similar way as the natural catalyst HLA-DM. Several structural and computational studies have been carried out to describe the mechanism of this transition on the molecular level. Moreover, *in vivo* studies in experimental cancer models demonstrated that the addition of MLE compound to the adjuvant greatly improves the antigen-specific CD4+ T cell response. MLE compounds may therefore be useful molecular tools to amplify immune responses during vaccination or therapy.

Selected publications (2006 to present)

Kleinewietfeld, M., Starke, M., Di Mitri, D., Borsellino, G., Battistini, L., Rotzschke, O., and Falk, K. (2009). CD49d provides access to "untouched" human Foxp3+ Treg free of contaminating effector cells. Blood 113, 827-836.

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Piaggio, E., Mars, L. T., Cassan, C., Cabarrocas, J., Hofstatter, M., Desbois, S., Bergereau, E., Rotzschke, O., Falk, K., and Liblau, R. S. (2007). Multimerized T cell epitopes protect from experimental autoimmune diabetes by inducing dominant tolerance. Proc Natl Acad Sci U S A 104, 9393-9398.

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Hopner, S., Dickhaut, K., Hofstatter, M., Kramer, H., Ruckerl, D., Soderhall, J. A., Gupta, S., Marin-Esteban, V., Kuhne, R., Freund, C., et al. (2006). Small organic compounds enhance antigen loading of class II major histocompatibility complex proteins by targeting the polymorphic P1 pocket. J Biol Chem 281, 38535-38542.

Patents (2005 to present)

PCT/EP2005/010008 - „Änderung des Beladungszustands von MHC Molekülen"

PCT/EP2008/008599 – "Method and kit for the rapid isolation of human Foxp3+ Treg cells"

PCT/EP2008/008660 - "Fusion protein comprising S-antigen repeat units"

Invention disclosure (07.05.2007): "Use of peptide derivates as MHC loading enhancers (MLE)"

Iduna Fichtner

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Experimental Pharmacology

Personalized medicine will be the future goal of treatment options in cancer therapy. To achieve this, the routine use of biomarkers for the prediction of response or inherited resistance of an individual tumor is necessary. Patient-derived tumor grafts gain increasing interest for the definition, characterization and validation of relevant biomarkers and are especially suitable models to investigate the dynamic regulation of genes or proteins in a clinically related way. The group "Experimental Pharmacology" has established a variety of patient-derived models from breast, colon, lung, ovarian carcinomas and from leukemias and sarcomas. Recent research was mainly focused on non-small cell lung carcinomas and their biomarker profiling in relation to the response to classical or targeted therapies. Further interest of the group concentrated on the generation of nanoparticulate formulations with a potential influence on vascular metastasis or for the treatment of brain malignancies. The continued stem cell research focused on comparisons between adult haematopoietic and embryonic stem cells. Both in vitro and in vivo methods were used to evaluate the transdifferentiation potential of these both cell types. It could be shown that embryonic stem cells have a higher potential for differentiation into hepatic lineage cells than adult stem cells.

Potential of patient derived xenografts for the identification of biomarkers

As recently reported, our group in cooperation with the Evangelische Lungenklinik Berlin-Buch (Dr. Merk) was able to establish a panel of non-small cell lung cancer (NSCLC) xenografts by transplanting patient derived surgical specimens directly to immunodeficient mice and keeping them in low passages. These tumor grafts were characterized using the expression of epidermal growth factor receptor (EGFR) related markers on the genetic (Affymetrix profiling, mutational analysis) and protein levels. It could be shown that there was a high congruence between the original and the xenotransplanted tumor concerning histology, immunohistochemistry and gene expression pattern. The chemotherapeutic responsiveness of the tumor grafts to classical cytotoxic drugs (paclitaxel, gemcitabine, carboplatin, etoposide, vinorelbine) as well as towards targeted therapeutics (cetuximab, erlotinib) resembled the clinical situation. No correlation could be found between mutations in the EGFR, p53, c-met or PI3K and response to therapy. However, a correlation between K-ras mutations and response to erlotinib was registered. After treatment with Cetuximab, a down-regulation of EGFR was observed in some sensitive but never in the resistant tumor models. The lack of therapeutic response of the NSCLC xenografts was not related to the expression of resistance markers like BCRP, LRP, MDR1 and MRP1 at RNA or protein level.

The comparison of the genome-wide gene expression profiling revealed a close clustering between the original patient tumor and the derived xenografts with correlation coefficients between 0.945 and 0.782 (Figure 1). 193 probe sets were differentially expressed. From

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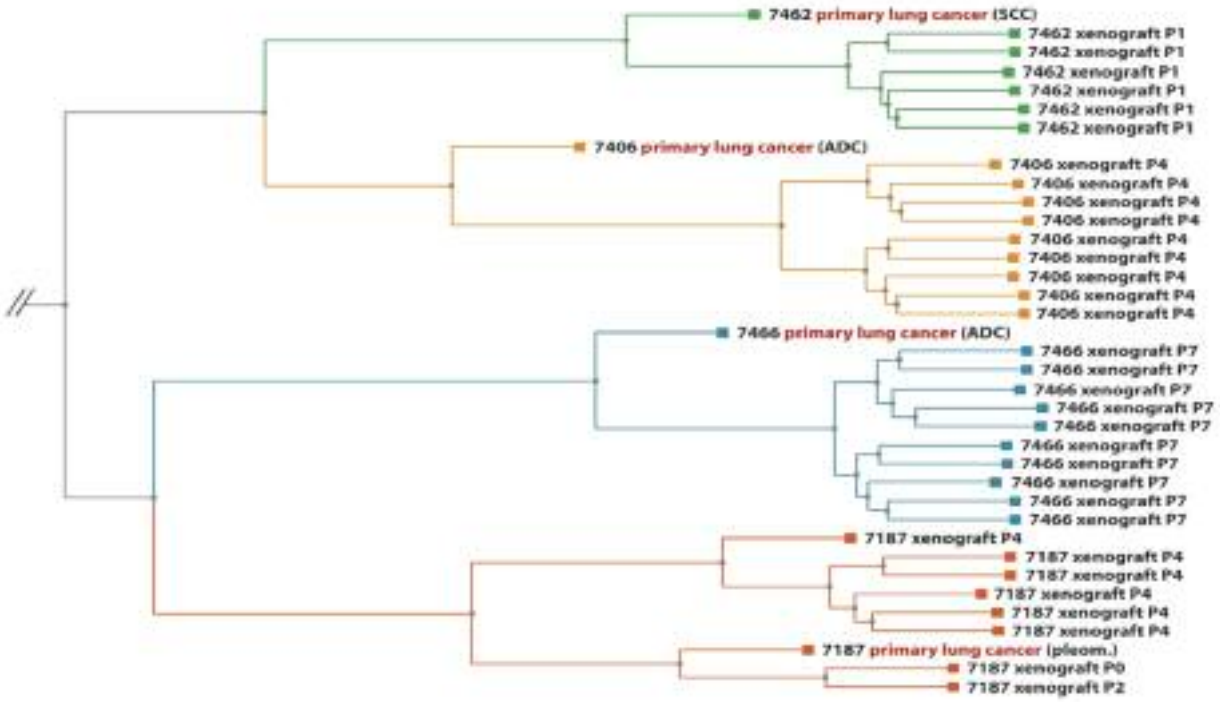


Figure 1. *One dimensional hirarchical clustering of primary lung carcinomas and their derived xenografts (Affymetrix profiling).*

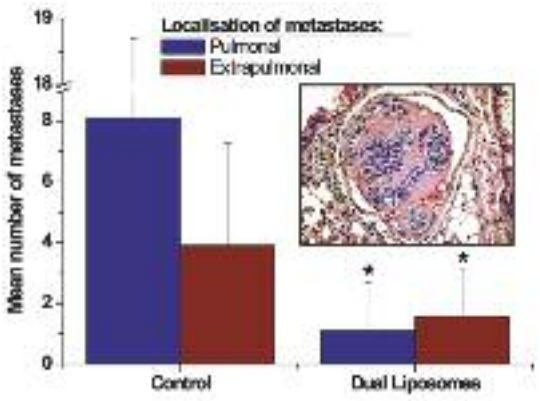
these, 155 probe sets mainly belonging to cell adhesion, immune response and extracellular pathways were down regulated. These data confirm the observation that during early xenotransplantation, human normal accessory cells are replaced by murine tissue while the tumors themselves maintain their identity. Some of the NSCLC xenografts were used as intracranial growing models to show the potential of a novel epothilone derivative, Sagopilone, to inhibit brain metastases in a very efficient way. Further analyses concerning the relevance of EGFR related signaling for response of tumors to anti-EGFR therapies are ongoing.

Inhibition of metastasis by nanoparticles

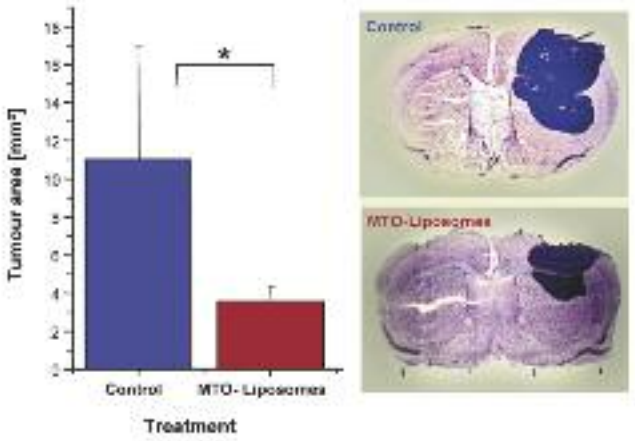
The formation of metastases is not completely understood and is the main reason for a failure in cancer therapy. We used the human MT3 breast cancer in a mouse xenograft model to gain a deeper insight into the mechanisms of metastasis. We found that the development of lung metastases is essentially dependent on the formation of fibrin clots in the vasculature as a precondition for a pulmonary arrest of tumor cells. Subsequently, intravascular micro-metastases are generated which finally invade into the surrounding tissue. We could further demonstrate a prevention of fibrin clot formation in vivo by the use of dual liposomes, simultaneously encapsulating the anticancer drug, Perifosine, and the haemostatic inhibitor, Dipyridamole. These dual liposomes are potent inhibitors of metastasis and caused a significant reduction in the number of lung metastases both in an experimental (MT3) (Figure 2 a) and in a spontaneous murine 4T7 breast carcinoma model. Our data suggest that these dual liposomes may display a new principle to prevent metastasis.

Figure 2

2a



2b



2a: *Dual liposomes significantly reduced the number of metastases of human MT-3 breast cancer in nude mice by preventing the formation of fibrin clots with proliferating tumor cells in a lung capillary (Micrograph: blue: tumor cells, red: fibrin).*

2b: *Mitoxantrone (MTO) loaded liposomes significantly (*) inhibited the intracerebral growth of MT-3 tumor (blue area in micrographs) after intravenous treatment, indicating a better transcellular transport across the blood-brain barrier.*

Liposomes were also developed to inhibit metastasis in the brain. Endothelial and epithelial barriers play an important role in the drug exchange between blood and tissues. Such tight cellular linings especially limit the drug transport across the blood brain barrier. Liposomes were designed to be used as drug transporters into the brain. They were characterized concerning their membrane properties, their uptake by and their transcytotic transport across an epithelial barrier in vitro and were tested concerning their therapeutic effect on breast cancer metastasis in the brain. Electron paramagnetic resonance measurements revealed a clear correlation between membrane fluidity of the liposomal bilayer and transcytosis. Liposomes with the helper lipids DOPE and Perifosine showed the highest transcytosis across a tight monolayer resulting in a five-fold increased transport of a marker into the basal medium of a transwell system in comparison to control liposomes. Liposomes containing both the helper lipids and Mitoxantrone as cytotoxic drug were shown to significantly inhibit tumor growth in the brain of nude mice by 61 % (Figure 2 b). It is expected that transcellular transport across the blood brain barrier can further be enhanced by fluid liposomes conjugated with a peptide for an active targeting to a specific receptor at the blood brain barrier. This project is currently ongoing.

Stem cell differentiation and transplantation into mouse models

Experimental and clinical studies showed that the transplantation of different stem cell types can contribute to the regeneration of injured tissues. The aim of our work was the evaluation and comparison of the spontaneous and directed hepatic differentiation of adult $CD34^+$ cord blood stem cells and human embryonic stem cells.

Human embryonic SA002 stem cells showed spontaneous in vitro differentiation into mesodermal, endodermal and ectodermal cell types. Hepatocyte conditioned medium and co-culture systems with murine hepatocytes were investigated regarding their potential to induce stem cell differentiation. Gene expression pattern of cultivated $CD34^+$ cells in hepatocyte conditioned medium resulted in an only moderate regulation of genes associated with cell cycle and cytoskeleton organization. In contrast to the restricted transdifferentiation potential of $CD34^+$ cells, SA002 cells in co-culture

with murine hepatocytes showed morphologic similarities to hepatic progenitor cells and revealed high levels of early endodermal and hepatic markers, measured by the detection of AFP, SOX17, HNF4 and albumin transcripts.

The in vivo properties of transplanted stem cells were examined regarding proliferation, engraftment and cell differentiation after application into newborn and adult immunodeficient mice. Intrahepatic transplantation of undifferentiated embryonic stem cells into mice resulted in fast and multiple teratoma growth. Different liver injury models were established in NOD/SCID mice and characterized with biochemical methods. An increase of hepatic engraftment after $CD34^+$ cell transplantation was determined in a model of chemically-induced liver injury.

Transplanted human embryonic stem cells into immunodeficient mice differentiated into cells of the three germ layers, including endodermal derived cells, as revealed by real-time RT-PCR with marker expression for AFP, HNF3/4 alpha and CK19 as well as by histology. Furthermore, gene expression pattern demonstrated that 1053 genes were up regulated more than 2 fold in the teratomas compared to undifferentiated embryonic stem cells, including genes for neuronal development as well as for angiogenesis. The teratomas showed a high degree of similarity to those structures of embryonic stem cells cultivated and differentiated in 3-D perfusion bioreactors (cooperation with Charite Berlin).

The established transplantation models are suitable for examining the organ distribution and the regenerative potential of stem cells.

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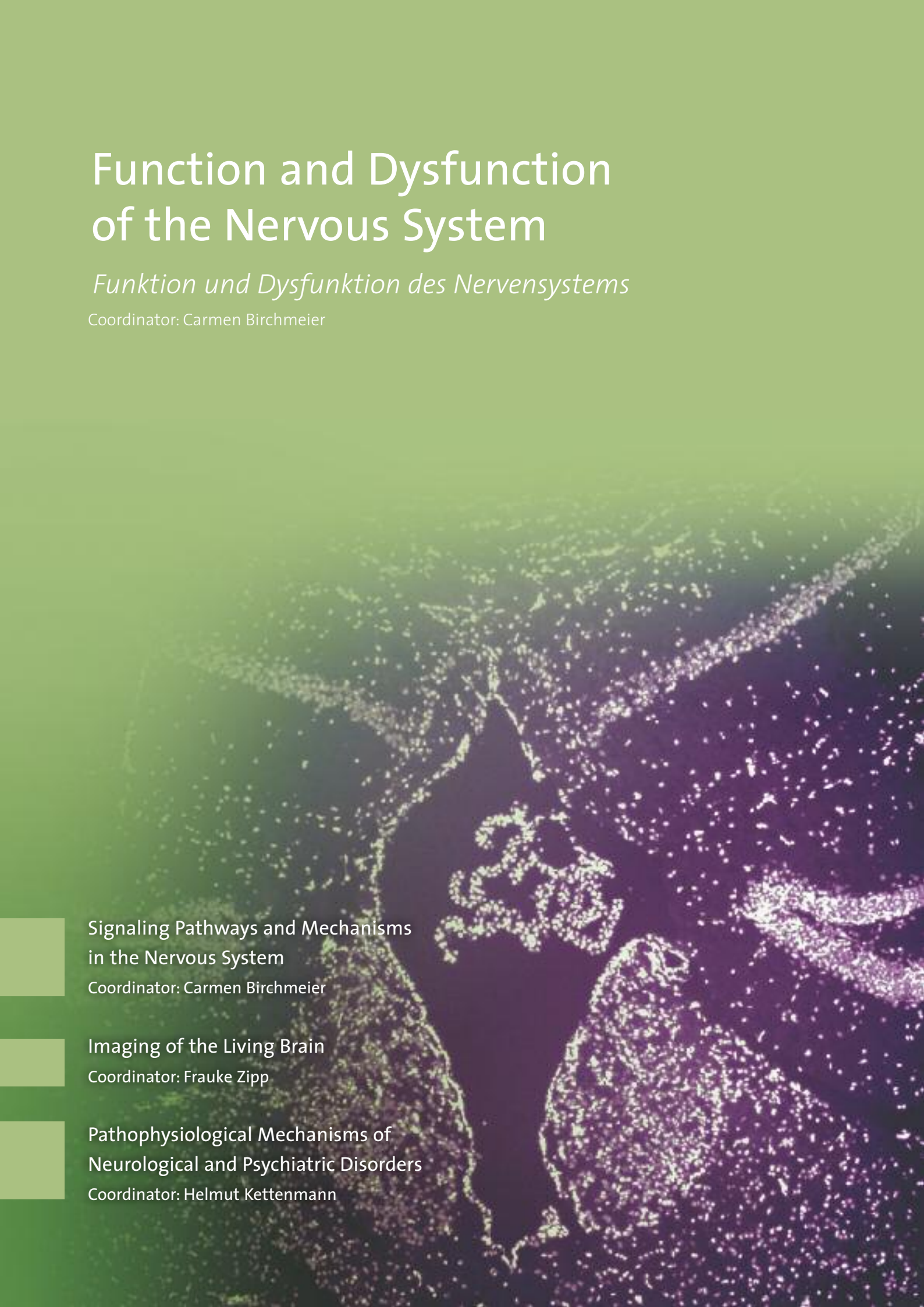
Function and Dysfunction of the Nervous System

Funktion und Dysfunktion des Nervensystems

Coordinator: Carmen Birchmeier



Signaling Pathways and Mechanisms in the Nervous System

Coordinator: Carmen Birchmeier

Imaging of the Living Brain

Coordinator: Frauke Zipp

Pathophysiological Mechanisms of Neurological and Psychiatric Disorders

Coordinator: Helmut Kettenmann

Function and Dysfunction of the Nervous System

Funktion und Dysfunktion des Nervensystems

The nervous system is a complex, sophisticated network that regulates and coordinates the body's basic functions and activities. Disorders of the nervous system that manifest themselves as neurological and psychiatric disease constitute a major challenge for affected individuals, their families, and for society. Determining the molecular basis of normal nervous system function, and discovering the changes responsible for inherited or acquired defects are important priorities in the fight against nervous system disease.

Research in the Neuroscience Department of the MDC focuses on molecular and cellular analysis of the central and peripheral nervous system. Themes and expertise covered by the department are broad and interdisciplinary, and comprise genetics, neurophysiology, proteomics and system biology, biochemistry, cell biology and stem cell research. This provides many opportunities for interactions and collaborations, and ensures an optimal environment for young researchers and group leaders. We are pleased that we were able to recruit three outstanding young scientist to the department during the past report period. **Björn Schroeder** joined the neuroscience department in 2008. He trained as a postdoc in the laboratory of Lily Jan und Yuh Nung Jan at the University of California San Francisco, where he used expression cloning to identify the first member of a family of calcium-activated chloride channels. Electrophysiological experiments had predicted the existence of such channels, but their molecular nature was not known until Björn developed a sophisticated assay that allowed their identification. **James Poulet** and **Jan Siemens** joined the department in 2009. James had worked at the Ecole Polytechnique Fédérale de Lausanne in the laboratory of Carl Petersen. He uses demanding and newly developed techniques that allow high-resolution recording of brain activity in awake and behaving animals. Jan trained in the laboratory of David Julius at the University of California in San Francisco. He identified new ion channel molecules that allow the detection of painful and cold stimuli by sensory neurons in the skin. He received the Sofia Kovalevskaya award in 2008, a prestigious prize granted by the Alexander von Humboldt Foundation, that will fund his work at the MDC during the next five years.

The SFB-TRR43 began its work in January 2008, and intensifies interactions and collaborations between researchers from Berlin and Göttingen. It devotes itself to the characterization of inflammatory mechanisms in central nervous system disease, and is entitled 'The Brain as a Target of Inflammatory Processes'. **Frauke Zipp** is the spokesperson of the SFB-TRR that includes 15 projects and

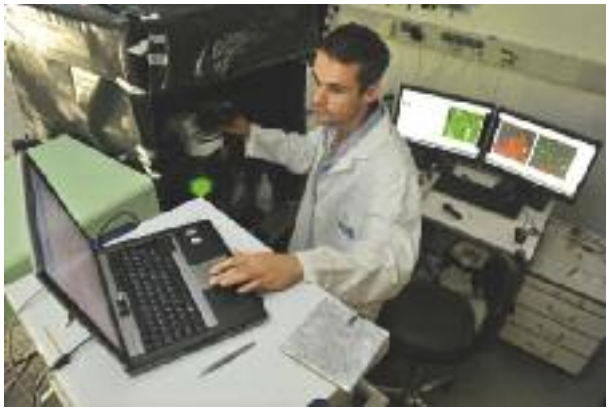
Das Nervensystem ist ein hochkomplexes Netzwerk, das die grundlegenden Funktionen und Aktionen des Körpers koordiniert. Störungen führen zu neurologischen und psychischen Krankheiten, die für die betroffenen Menschen, ihre Familien und die ganze Gesellschaft ein erhebliches Problem darstellen. Die Untersuchung der molekularen Grundlagen der normalen Nervenfunktion und die Aufklärung der Ursachen für ererbte oder erworbene Störungen des Nervensystems sind von herausragender Bedeutung im Kampf gegen solche Krankheiten.

Die Forschung im Fachbereich "Neurowissenschaft" des MDC konzentriert sich auf die molekulare und zellbiologische Analyse des zentralen und des peripheren Nervensystems. Forschungsthemen und -methoden, die im Bereich behandelt werden, sind interdisziplinär breit ausgerichtet und umfassen Genetik, Neurophysiologie, Proteomik, Systembiologie, Zellbiologie und Stammzellforschung. Dies bietet zahlreiche Kooperationsmöglichkeiten und ermöglicht eine optimale Forschungsumgebung für junge Forscher und Forschungsgruppenleiter.

Wir hatten das Glück, in der Berichtsperiode drei talentierte junge Forscher in den Bereich zu integrieren. ***Björn Schroeder*** *kam im Jahr 2008 an das MDC. Er wurde als PostDoc im Labor von Lily Jan und Yuh Nung Jan an der Universität von Californien in San Francisco ausgebildet. Mittels Expressionsklonierung gelang ihm die Identifizierung des ersten Mitglieds einer Familie von kalziumaktivierten Chlorid-Ionenkanälen. Elektrophysiologische Messungen hatten die Existenz solcher Kanäle vermuten lassen, aber ihre molekulare Natur war unbekannt, bis Björn sie mit einem komplizierten Nachweisverfahren identifizieren konnte.* ***James Poulet*** *und* ***Jan Siemens*** *kamen 2009 in den Fachbereich. James hatte zuvor an der Ecole Polytechnique Fédérale de Lausanne im Labor von Carl Petersen gearbeitet. Er arbeitet mit neuentwickelten aufwendigen Techniken daran, die Hirnaktivität in wachen, sich spontan verhaltenden Tieren in hoher Auflösung zu erfassen. Jan wurde in der Arbeitsgruppe von David Julius an der Universität von Californien in San Francisco ausgebildet. Er fand neue Ionenkanalmoleküle, die die Entdeckung von Schmerz- und Kältereizen durch sensible Neuronen in der Haut ermöglichen. Jan wurde 2008 mit dem Sofya-Kovalevskaya-Preis ausgezeichnet, einem angesehenen Preis der Alexander-von-Humboldt-Stiftung, der ihm die Finanzierung seiner Arbeit am MDC für die nächsten fünf Jahre sichert.*

Der Sonderforschungsbereich SFB-TRR43 begann im Januar 2008 mit seiner Arbeit. Unter dem Titel "Das Gehirn als Ziel entzündlicher Prozesse" intensiviert er die Zusammenarbeit von Forschern in Berlin und Göttingen bei der Charakterisie-

Photo: David Ausserhofer / Copyright: MDC



Dr. Volker Siffrin (a medical doctor in training, research group: Prof. Frauke Zipp) uses the Two-Photon-Laser-Scanning-Microscope to perform intravital imaging of the brainstem of a mouse with chronic inflammation of the central nervous system.

Dr. Volker Siffrin (ein Arzt in der Ausbildung, Forschungsgruppe: Prof. Frauke Zipp) verwendet das Zwei-Photon-Laser-Scanning-Mikroskop um eine intravitale Darstellung des Gehirnstamms einer Maus mit chronischer Entzündung des zentralen Nervensystems zu erzeugen.

is funded by the German Science Foundation with 2.5 Mio €/year. In 2008 Thomas Jentsch was awarded a collaborative research grant entitled "Physiology and Pathology of KCNQ potassium channels" worth ~1 mio € from the Leibnitz society as part of its "Pakt für Forschung". Grant funding started in 2009 and the grant supports a multidisciplinary examination of KCNQ in collaboration with Gary Lewin and Dietmar Schmitz (Charité, Berlin). **Carmen Birchmeier** successfully established a research network that is funded by the Ministry of Research and Education, which devotes itself to the analysis of the stem cells of the adult muscle (SatNet). Further researcher participating in the network are Nikolaus Rajewsky (MDC) Simone Spuler and Markus Schülke-Gerstenfeld (Charité, Berlin) and Thomas Braun (MPI Bad Nauheim).

During the report period, **Helmut Kettenmann** was elected president of the Federation of European Neuroscience Societies, and he became also a member of the Academia Europaea. Also, **Gary Lewin** was elected as a new member of EMBO in 2008. **Erich Wanker** and members of his research team were awarded the Erwin Schrödinger Prize in 2008 by the Helmholtz Association of German Research Centers for their work on neuroproteomics and the generation of a first human protein-protein interaction map. Erich Wanker's group began to set up a new platform named *'Interactome'* funded by the Ministry of Research and Education via an extension of the NGFN. This platform will use new high throughput technologies to analyze and validate protein interaction data.

rung entzündlicher Prozesse in Krankheiten des Zentralen Nervensystems. ***Frauke Zipp*** *ist die Sprecherin des SFB-TRR, der 15 Projekte vereint und von der Deutschen Forschungsgemeinschaft mit 2.5 Mio Euro/Jahrfinanziert wird.*

Thomas Jentsch *erhielt 2008 von der Leibnitz Gesellschaft eine Forschungszuwendung von 1 Mio Euro für das Verbundprojekt "Physiologie und Pathologie der KCNQ Kaliumkanäle". Die Förderung lief 2009 an, und unterstützt eine multidisziplinäre Untersuchung der KCNQ Kanäle in Zusammenarbeit mit Gary Lewin und Dietmar Schmitz (Charité Hochschulmedizin Berlin).* ***Carmen Birchmeier*** *etablierte 2008 ein Verbundprojekt zu Stammzellen der Skelettmuskulatur, SatNet, das vom Bundesministeriums für Bildung und Forschung gefördert wird; an dem Projekt partizipieren neben Nikolaus Rajewsky (MDC) auch Wissenschaftler der Charité (Simone Spuler und Markus Schülke-Gerstenfeld) und der Max-Planck-Gesellschaft (Thomas Braun, Bad Nauheim).*

Während der Berichtsperiode wurde ***Helmut Kettenmann*** *zum Präsidenten der Föderation der Europäischen Neurowissenschaftlichen Gesellschaften gewählt, und zum Mitglied der Academia Europaea berufen. Ebenfalls 2008 wurde* ***Gary Lewin*** *als neues EMBO Mitglied gewählt.*

Erich Wanker *und Mitglieder seine Arbeitsgruppe erhielten 2008 den Erwin Schrödinger Preis der Helmholtz-Gemeinschaft deutscher Forschungszentren für ihre Arbeit auf dem Gebiet der Neuroproteomik und die Erstellung der ersten menschlichen Protein-Protein Interaktionskarte. Die Arbeitsgruppe erhielt zudem erhebliche Drittmittel des Bundesministeriums für Bildung und Forschung für die Entwicklung einer neuen „Interactom"-Plattform im Rahmen des NGFN-Projekts. Diese Plattform wird Hochdurchsatztechnologien zum Nachweis von Proteininteraktionen einsetzen.*

Während des Berichtszeitraums hat der Fachbereich "Neurowissenschaften" unter der Federführung von Gary Lewin die Helmholtz International Research School "Molecular Neurobiology" etabliert. Es handelt sich dabei um ein gemeinsames Programm des MDC mit der Freien Universität und der Charité, das Doktoranden ausbilden soll, die an der Analyse neurobiologischer Prozesse arbeiten. Erfolgreiche Bewerber erhalten vollfinanzierte Doktorandenstellen an einer der beitragenden Institutionen, um an einem Projekt zu den molekularen Grundlagen von Funktion und Dysfunktion des Nervensystems zu arbeiten. Der Lehrplan des Programms beinhaltet einen zweijährigen Vorlesungszyklus über grundlegende sowie fortgeschrittene Konzepte der Neurobiologie, einen Journal Club der Studenten, und prak-

The Neuroscience Department also established the Helmholtz International Research School 'Molecular Neurobiology'. This program is a joint activity of the Max-Delbrück Centre (MDC) for Molecular Medicine, the Free University Berlin, and the Charité, and is headed by Gary Lewin. The aim of the school is to provide state of the art training to PhD students working on the analysis of neurobiological processes. Successful PhD applicants are offered a fully funded doctoral position either at the MDC, the Free University or the Charité, and they are expected to pursue a research project that devotes itself to the molecular understanding of normal function and dysfunction in the nervous system. The curriculum of the program includes a two years lectures series covering basic and advanced concepts of neurobiology, a student journal club, and practical experimental courses. In addition, the program offers 'soft skills' training, for instance courses on communication and presentation, and funding to allow students to attend international conferences to present their results.

Activities in the department include a biannual retreat that is attended by students, postdocs and group leaders. In 2009, the retreat took place in a small village in Brandenburg, Hubertusstock. The beautiful and secluded environment there was well suited for intense but informal scientific discussion and presentation of results.

Signaling pathways and mechanisms in the nervous system

Signaling systems control the establishment of gene expression programs and cellular interactions during development. Their analysis provides insight into the mechanisms used to build the nervous system, and unravels the cause of developmental disorders that underlie disease. In the mature nervous system, signaling molecules control synaptic functions or neuronal maintenance, are key molecules in neuron-glia interactions, and control the transmission and processing of electrical information. Many groups in the Neuroscience Department focus on defining the roles of signaling molecules in the developing and adult nervous system, in the healthy as well as in the afflicted organism.

Human Bartter syndrome type IV is associated with severe renal loss of fluid and salt as well as with congenital deafness and is caused by mutations in the BSND gene. The group of **Thomas Jentsch** has shown previously that barttin, the protein encoded by BSND, is an accessory -subunit of the chloride channels ClC-Ka and –b. Both channels are expressed in the kidney and in the cochlear

tische Kurse. Überdies werden Kommunikations- und Präsentationstechniken erlernt und Stipendien für die Teilnahme an internationalen Tagungen gewährt, auf denen die Studenten ihre Ergebnisse präsentieren können.

Eine weitere Fortbildungsaktivität sind die regelmäßigen Klausurtagungen, die im Jahre 2009 Studenten, PostDocs und Gruppenleiter im Brandenburger Ort Hubertusstock zusammenbrachte. Die abgelegene, landschaftlich schöne Umgebung bot einen geeigneten Rahmen für intensive informelle Diskussion und Vorstellung der neuesten Ergebnisse.

Signalwege und Signalmechanismen im Nervensystem

Signalsysteme kontrollieren Genexpressionsprogramme und zelluläre Interaktionen während der embryonalen Entwicklung. Ihre Analyse gewährt Einsicht in die Mechanismen der Entstehung des Nervensystems und hilft, Entwicklungsstörungen zu verstehen, die zu neurologischen Erkrankungen führen. Im ausgereiften Nervensystem kontrollieren Signalmoleküle die synaptische Funktion und die Aufrechterhaltung der neuronalen Strukturen. Sie sind die Schlüsselmoleküle für die Interaktion von Neuronen und Gliazellen und steuern die Übertragung und Weiterverarbeitung elektrischer Signale. Etliche Gruppen im Fachbereich konzentrieren sich auf die Rolle, die Signalmoleküle im sich entwickelnden und im adulten Nervensystem spielen, sowohl im gesunden wie im pathologisch veränderten Organismus.

Das klinische Bartter Syndrom des Typs IV ist gekennzeichnet durch erhebliche Flüssigkeits- und Salzverluste über die Niere zusammen mit angeborener Taubheit, und wird durch Mutationen im BSND-Gen verursacht. Die Gruppe um ***Thomas Jentsch*** *hatte bereits gezeigt, dass das von diesem Gen kodierte Protein Barttin eine zusätzliche Beta-Untereinheit der Chloridkanäle CIC-Ka und CIC-Kb bildet. Beide Kanäle sind sowohl in der Niere wie im Cochlear-Epithel der stria vascularis des Innenohrs exprimiert.*

Die Forscher stellten nun eine konditionelle "gefloxte" barttin Mutante in der Maus her, mit der das Gen gezielt nur im Innenohr deletiert wurde, um so die durch Salz- und Flüssigkeitsverlust verursachte postnatale Letalität der konventionellen barttin Nullmutante zu vermeiden. Diese Tiere sind genau wie Patienten mit einer BSND-Mutation angeboren taub. Die Wissenschaftler in der Arbeitgruppe von Thomas Jentsch konnten zeigen, dass dies auf einen Kollaps des elektropositiven Potentials in der scala media (dem sog. endocochlearen Potential) zurückzuführen ist. Letzteres ist essentiell, um einen depolarisierenden Kalium-Einstroms durch

epithelium of the stria vascularis in the inner ear. They now generated a conditional 'floxed' barttin KO mouse to delete barttin exclusively in the inner ear, thereby avoiding the massive salt and fluid loss that leads to early postnatal death of mice carrying null-mutations in BSND gene. Like patients with mutations of BSND, these mice are congenitally deaf. They showed that this is due to a collapse of the positive voltage in the scala media (the endocochlear potential), which is necessary to drive a depolarizing influx of potassium through mechanosensitive channels in sensory hair cells. Outer hair cells were no longer able to mechanically amplify sound in the inner ear and eventually degenerated. The group has thus clarified the pathological mechanism underlying a form of human deafness and revealed a previously unknown role of chloride channels in the generation of the endocochlear potential (Rickheit et al., EMBO J. 27, 2907-2917).

In a healthy organism, a balance is maintained between the excitation and inhibition of electrical neuronal impulses. Deregulation of this balance results in nervous system disorders. The group of **Jochen Meier** investigates post-transcriptional processes that control the balance between excitation and inhibition. Glycine receptors are ligand-gated ion channels that recognize the amino acid glycine, which acts as an inhibitory neurotransmitter. Jochen had previously discovered that mRNA encoding for the glycine receptor is post-transcriptionally modified: the modified mRNA encodes a receptor that binds glycine with a higher affinity than the receptor encoded by the unmodified RNA. The group currently characterizes the compensatory function of these high affinity receptors in hyperexcitability disorders.

The group of **Ines Ibanez-Tallon** has developed a novel genetic method to manipulate neuronal activity in vivo using genetically encoded cell-surface anchored toxins and neuropeptides. These toxins interfere with ion channel function and are designed to silence neurons via inhibition of ion channels in a cell-autonomous manner, so that only the neuron that expresses the toxin is affected. The design of these tethered toxins follows nature, as endogenous molecules exist in mammals that modulate neuronal activity in such a manner (Tekinay et al., PNAS 2009). The group of Ines Ibanez-Tallon does use these novel tools to define the function of a particular neuron in a neuronal circuit, or to characterize the function of a class of ion channels in a specific neuronal population.

During his postdoc years, **Björn Schroeder** identified a prototype of a new family of ion channel (Schroeder et al., 2008, Cell). Two members of this family, TMEM16A and TMEM16B, encode calcium activated chloride channels. In

die mechanosensitiven Kanäle der sensorischen Haarzellen zu treiben. Die äußeren Haarzellen sind so nicht mehr in der Lage, den eingehenden Schall im Innenohr zu verstärken, und degenerieren schließlich. Mit diesen Arbeiten hat Jentschs Gruppe einen Pathomechanismus für menschliche Taubheit aufgeklärt und eine zuvor unbekannte Rolle von Chloridkanäle bei der Herstellung des endochochlearen Potentials aufgedeckt (Rickheit et al., EMBO J. 27, 2907-2917).

Im gesunden Organismus besteht ein Gleichgewicht zwischen exzitatorischen und inhibitorischen Nervenimpulsen. Störungen dieses Gleichgewichts führen zu Erkrankungen des Nervensystems. Die Gruppe von ***Jochen Meier*** *untersucht die post-transkriptionellen Prozesse, die das Gleichgewicht aufrecht erhalten. Glycinrezeptoren sind Ligandenaktivierte Ionenkanäle, die die Aminosäure Glyzin binden, welche hier als hemmender Neurotransmitter funktioniert. Jochen Meier konnte bereits zeigen, dass mRNA, die für diesen Rezeptor kodiert, post-transkriptionell modifiziert wird: die modifizierte mRNA kodiert für einen Rezeptor, der eine höhere Affinität zu Glyzin aufweist. Die Arbeitsgruppe untersucht nun die kompensatorische Funktion des hochaffinen Rezeptors bei hyper–exzitatorischen Störungen.*

Der Arbeitsgruppe von ***Ines Ibanez-Tallon*** *gelang es, neuronale Aktivität in vivo mit Hilfe genetisch kodierter, zelloberflächengebundener Toxine und Neuropeptiden zu manipulieren. Diese Toxine interferieren mit Ionenkanälen und sind so gestaltet, dass sie zellautonom nur diejenigen Neuronen funktionell beeinträchtigen, von denen sie exprimiert werden. Das Design dieser oberflächengebundenen Toxine ist dem von endogenen Modulatoren neuronaler Funktion ähnlich (Tekinay et al., PNAS 2009). Die Gruppe um Ibanez-Tallon nutzt diese neuartigen Moleküle als Werkzeuge, um die Funktion einer bestimmten Nervenzelle in einem neuronalen Schaltkreis zu bestimmen, oder um die Funktion einer Klasse von Ionenkanälen in einer bestimmten Populationen von Neuronen aufzuklären (Auer et al., Nature Methods, 2010, in press).*

Während seiner Jahre als PostDoc gelang es ***Björn Schroeder****, den Prototyp einer neuen Ionenkanal-Familie zu entdecken (Schroeder et al., 2008, Cell). Zwei bestimmte Gene dieser Familie, TMEM16A und TMEM16B, kodieren für kalzium-aktivierbare Chloridkanäle. Schroeder will die Funktion dieser Kanäle mit Hilfe von genetischen Experimenten in der Maus untersuchen. Besonders interessiert ihn dabei die Rolle solcher Kanäle bei Geruchs- und Geschmacksempfindungen und bei der Blutdruckregulation, sowie beim transepithelialen Wasser- und Ionentransport in der Lunge. Beabsichtigt sind auch strukturelle und funktionelle Studien an diesen Kanälen mit Hilfe der sog. site-directed mutagenesis in einem transienten Expressionssystem.*

the coming years, Björn Schröder plans to functionally characterize these channels using mouse genetics. In particular, he intends to determine the role of these channels in olfaction, taste and blood pressure regulation, and is interested in their activity during transepithelial water and ion transport in the lung. In addition, he will perform structural and functional studies on these novel channels using site-directed mutagenesis and a transient expression system.

The ability of any living organism to sense stimuli emanating from the surrounding environment is of fundamental importance for its well-being and survival. The somatosensory system allows the detection of pain and mechanical stimuli as well as changes in temperature. Sensory mechanisms not only monitor the outside world but also detect changes of interior parameters such as blood sugar levels, blood pressure or body temperature. Processing and integration of sensory information from the outside world and the interior environment allows our body to adjust to changes and to maintain homeostasis.

The naked mole rat, also known as desert mole rat, is an unusual rodent that thrives in a harsh and hot underground environment. It is the only known poikilothermic mammal (i. e. 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). This animal has a normal acute pain response, but displays no hypersensitivity (so called hyperalgesia) to a variety of inflammatory and chemical stimuli. Strikingly, the animals lack behavioral response to acid. The group of **Gary Lewin** has used electrophysiology to show that primary afferent nociceptors in naked mole-rats are insensitive to acid stimuli, consistent with the lack of acid-induced behavior. Acid sensitivity by sensory neurons is observed in birds, amphibians, and fish, which suggests that it has been selectively lost in the naked mole-rat in the course of its evolution. In contrast, nociceptors of the naked mole rate do respond vigorously to capsaicin, a pain-inducing chemical isolated from chili peppers. Nevertheless, the activation of capsaicin-sensitive sensory neurons in naked mole-rats does not produce pain-related behavior. The unique pain response of the naked mole-rat can provide insights into what constitutes normal mammalian nociception. To elucidate its cause, the Lewin group is currently analyzing gene variants of the naked mole rat that encode ion channels and associated channels that are required for the transduction of painful stimuli

Jan Siemens found that three inhibitor cysteine knot (ICK) peptides from tarantula venom target a particular TRP ion channel, TRPV1. TRPV1 functions also as the receptor for

Die Registrierung von Reizen aus der Umgebung ist eine essentielle Funktion für Wohlbefinden und Überlebensfähigkeit eines jeden Organismus. Das somatosensorische System vermittelt dabei die Wahrnehmung von mechanischen und Schmerz-Reizen sowie die Empfindung für die Umgebungstemperatur. Sensorische Mechanismen registrieren jedoch nicht nur äußere Einflüsse, sondern auch innere Parameter wie Blutzuckerniveau, Blutdruck oder die Körpertemperatur. Beide Wahrnehmungsvarianten erlauben dem Körper die gezielte Anpassung an Veränderungen und die Aufrechterhaltung der Homöostase des Organismus.

Das Nacktmull, eine Rattenart, die auch als Wüstenmull bezeichnet wird, ist ein sehr ungewöhnliches Nagetier, das in unwirtlichen und heißen Böden lebt. Es ist das einzige bisher bekannte wechselwarme Säugetier, lebt in insektenähnlichen sozialen Strukturen und ist die Nagerart mit der größten Lebenserwartung (mehr als 25 Jahre). Dieses Tier zeigt eine normale akute Schmerzempfindlichkeit, ist jedoch nicht überempfindlich (hyperalgesisch) gegen eine Reihe chemischer und entzündungserzeugender Reize. Auffallend ist das Fehlen einer Reaktion auf Säure. Die Forschergruppe um ***Gary Lewin*** *nutzte elektrophysiologische Methoden, um zu zeigen, dass die primären afferenten Nozirezeptoren im Nacktmull nicht auf Säurestimuli reagieren, wie es bei Vögeln, Amphibien und Fischen der Fall ist. Das legt nahe, dass der Nacktmull eine analoge Funktion in der Evolution verloren hat. Hingegen wurde festgestellt, dass die Nozirezeptoren des Nacktmull heftig auf Capsaicin reagieren, eine schmerzauslösende Substanz, die aus Chillischoten isoliert wird. Allerdings erzeugt die Aktivierung der Capsaicin-empfindlichen sensorischen Neurone nicht das sonst übliche schmerzinduzierte Verhalten der Tiere. Diese einzigartige Schmerzreaktion im Nacktmull vermittelt indirekt Einsichten in die Funktionsweise der normalen Nozizeption von Säugetieren. Um den Mechanismus weiter aufzuklären, analysiert Lewin gegenwärtig Genvarianten des Nacktmulls, die für Ionenkanäle und assoziierte Kanäle kodieren und für die Transduktion von Schmerzreizen verantwortlich sind.*

Jan Siemens *hat festgestellt, dass drei sogenannte inhibitorische Cystein-Knoten-Peptide (ICK) aus dem Gift der Tarantel einen bestimmten TRP-Ionenkanal, TRPV1, angreifen. Dieser TRPV1 ist ein Capsaicin-Rezeptor, dessen Aktivierung einen brennenden Schmerz erzeugt. Die ICK-Toxine wirken als TRPV1-Agonisten, was auch erklärt, warum das Tarantelgift Schmerzen erzeugen kann. Damit liegt nun mit den ICK Toxinen ein neues pharmakologisches Werkzeug vor, um die Funktion von TRP Kanälen zu studieren. Zugleich eröffnen sich neue Ansätze zur Entwicklung von Medikamenten zur Therapie von Schmerzzuständen (Siemens et al., Nature*

capsaicin, the active component of chili peppers, which produces a burning and painful sensation. The ICK toxins function as TRPV1 agonists, which explains how the tarantula venoms can cause pain. The ICK toxins thus constitute new pharmacological tools to study TRP channel gating, and open new avenues to design drugs that block the pain pathway (Siemens et al., Nature. 444, 208-212). While we have learned a lot about biophysical properties of individual TRP ion channels over the last couple of years, little is known about their functional modulation and regulation *in vivo*. By analogy to TRP channels in the Drosophila eye, we hypothesize that the mammalian orthologs are components of supramolecular membrane-bound protein complexes that enable the channels to function specifically and effectively in a context dependent manner. Jan Siemens will use make use of genetic, biochemical and functional screening paradigms to identify components of this putative modulatory complex.

Sensory neurons project to the spinal cord, and innervate neurons located in the dorsal horn. Neurons in the dorsal horn of the spinal cord integrate and relay the sensory information to other parts of the nervous system. Sensory axonal projections into the spinal cord display a highly stereotyped pattern of T- or Y-shaped axon bifurcation at the dorsal root entry zone. The group of **Fritz Rathjen** identified molecules that control sensory axon bifurcation. These are the natriuretic peptide C (CNP), its receptor, the guanylyl cyclase Npr2, and its downstream signaling mediator, the cyclic guanosine monophosphate-dependent protein kinase I (cGKI). Bifurcation does not occur in mice with mutations in one of these components suggesting that they act in a signaling cascade. This is accompanied and by altered neuronal connectivity in the dorsal spinal cord, and by changes in the perception of painful stimuli (Schmidt et al., 2009, PNAS, in press).

A further relay station for sensory information is provided by hindbrain neurons. Somatosensory neurons innervate the trigeminal nucleus of the hindbrain and convey information about touch and pain from the face, whereas viscerosensory neurons innervate the nucleus of the solitary tract to convey information about parameters like visceral pain or blood pressure. The group **of Carmen Birchmeier** identified two transcription factors, Lbx1 and Olig3, that are essential for the development of these two hindbrain nuclei, and that appear to play opposing roles during their development. The homeobox gene Lbx1 is essential for generation of the trigeminal nucleus. In Lbx1 mutant mice, instead of a trigeminal nucleus, an extremely large nucleus of the solitary tract is formed. Conversely, the nucleus of the solitary tract is absent in Olig3 mutant

444,208-212). Während die biophysikalischen Eigenschaften einzelner TRP Kanäle in den letzten Jahren erforscht wurden, ist relativ wenig über ihre funktionale Modulation und Regulierung in vivo bekannt. Es wird vermutet, dass, analog zu den TRP Kanälen in den Augen von Drosophila, die Orthologe in Säugetieren ebenfalls Komponenten von membrangebundenen Proteinkomplexen sind, in deren Kontext die Kanäle spezifische Funktionen übernehmen. Jan Siemens wird genetische, biochemische und funktionelle Screeningverfahren einsetzen, um weitere Bestandteile dieser vermuteten modulatorischen Komplexe zu identifizieren.

Sensorische Neuronen leiten Impulse an das Rückenmark weiter und innervieren hier die Neuronen im Hinterhorn. Diese integrieren die Information und leiten sie weiter in andere Regionen des Nervensystems. Sensorische axonale Projektionen ins Rückenmark zeigen ein hoch stereotypes Muster von T- oder Y-förmigen Axonaufzweigungen an der Eintrittszone in die Wurzeln des Hinterhorns. Die Gruppe von ***Fritz Rathjen*** *hat Moleküle gefunden, die die sensorische Axonaufzweigung kontrollieren. Verantwortlich ist das natriuretischen Peptid C (CNP) mit seinem Rezeptor, der Guanylatzyklase Nrp2, und dem Signalvermittler, der Guanosinmonophosphat-abhängigen Proteinkinase I (cGKI). Zur Bifurkation kann es nicht kommen, wenn einer dieser Faktoren durch Mutation in der Maus inaktiviert ist, was auf ihre Verschaltung als Signalkaskade hinweist. In der Folge treten veränderte Nervenverschaltungen im dorsalen Rückenmark auf, und Änderungen in der Schmerzempfindung (Schmidt et al., 2009, PNAS, im Druck)*

Eine weitere Relaisstation für sensorische Information sind die Neuronen des Hirnstamms. Somatosensorische Neuronen innervieren den Trigeminuskern im Rautenhirn und vermitteln damit Information über Berührungs- und Schmerzreize im Gesicht, während viszerosensorische Neuronen den Geschmackskern des Nervus vagus (Nucleus tractus solitarii) mit Informationen über abdominale Schmerzempfindungen und Blutdruckvariation versorgen. Die Gruppe von ***Carmen Birchmeier*** *hat zwei Transkriptionsfaktoren, Lbx1 und Olig3, identifiziert, die für die embryonale Entwicklung dieser zwei Hirnstammkerne essentiell sind, und gegensätzliche Funktionen während der Ausbildung der Region übernehmen. Das Homöobox-Gen Lbx1 ist für die Ausbildung des Trigeminuskerns erforderlich. In mutanten Mäusen ohne Lbx1 bildet sich anstelle des Kerns ein extrem großer Solitartrakt aus; in Olig3-mutanten Mäusen hingegen fehlt der Solitartrakt. Eine genauere Analyse der Funktion von Olig3 zeigte, dass eine wichtige Funktion dieses Faktors in der Unterdrückung von Lbx1 in Hirnstammneuronen besteht (Storm et al., Development 2009, 139:295-305).*

mice. Further analysis of the function of Olig3 demonstrated that an important aspect of its developmental function lies in the suppression of Lbx1 in hindbrain neurons (Storm et al., Development 2009 136:295-305).

Recent technical advances have allowed researchers to make high-resolution recordings of brain activity even while animals are awake and behaving, which provides remarkable insights into normal brain function. Of particular interest is the activity of neurons in the neocortex, as they are involved in conscious movement and sensory perception. The group of **James Poulet** combines electrophysiological and optical neural recordings to investigate the link between neural activity, sensory perception and motor behavior. The very first recordings from the awake human brain by Hans Berger (1929) revealed distinct patterns of cortical activity, or "brain states" during rest as compared to motor activity. Using dual whole-cell recordings from layer 2/3 primary somatosensory mouse whisker barrel cortex, James Poulet is investigating the mechanisms and functions of changes in brain state. His initial recordings have shown that the intracellular membrane potential of neurons is highly synchronized while the mouse is sitting still. However, when the mouse moves its whiskers to sense the environment, a desynchronized local field potential and electroencephalogram are observed (Poulet and Peterson, Nature 2008). In the future James Poulet and his group will analyze the primary sensory and motor cortical areas controlling limb movement. Cortical activity controlling limb movement is particularly relevant in the emerging field of neuroprosthetics, where cortical activity from paralyzed patients is used to drive robotic limbs.

Imaging of the living brain and pathophysiological mechanism of neurological and psychiatric disorders

Disorders of the nervous system are a major challenge of our society. Several groups in the MDC Neuroscience Department concentrate on elucidating molecular mechanisms that lead to nervous system disease, thus aiming for the development of rational therapies, as for instance the design of small molecular therapeutical compounds.

Multiple sclerosis is an autoimmune disease in which the patient's immune response attacks the central nervous system, leading to demyelination.

Multiple sclerosis affects thus the ability of nerve cells to communicate with each other. The group of **Frauke Zipp** studies molecular mechanisms that cause multiple sclerosis, and define molecules that affect the pathophysio-

Neue technische Entwicklungen haben es möglich gemacht, die Hirnaktivität wacher und verhaltensaktiver Tiere mit hoher Empfindlichkeit zu registrieren., Von besonderem Interesse ist hierbei die Aktivität der Neuronen des Großhirns, denn sie sind sowohl an bewussten Bewegungen wie an der sensorischen Wahrnehmung der Umgebung beteiligt. Die Gruppe von ***James Poulet*** *nutzt elektrophysiologische und optische Ableitungen, um die Verbindung zwischen neuronaler Aktivität, sensorischer Wahrnehmung und motorischer Antwort zu studieren. Die ersten Ableitungen eines wachen menschlichen Gehirns durch Hans Berger (1929) enthüllten spezifische Muster kortikaler Aktivität, oder "Gehirnzustände", abhängig von Ruhezustand oder motorischer Aktivität. Mit Hilfe paralleler Ableitungen von Zellen aus den Schichten 2/3 des somatosensorischen Tasthaar-Barrel-Kortex der Maus untersucht James Poulet Mechanismus und Zweck von Änderungen des Gehirnstatus. Erste Ergebnisse zeigen, dass das intrazelluläre Membranpotential stark synchronisiert ist, solange eine Maus still sitzt. Bewegt die Maus hingegen die Tasthaare, um die Umgebung zu erkunden, dann desynchronisiert das lokale Feldpotential sowie das EEG (Poulet & Peterson, Nature 2008). Die Arbeitsgruppe will nun die Bereiche des primären sensorischen und motorischen Kortex analysieren, die die Extremitäten kontrollieren. Solche Arbeiten sind besonders relevanten für das aufkommende Forschungsfeld der Neuroprosthetik, wo kortikale Impulse von gelähmten Patienten zur Steuerung von mechanischen Gliedmaßen benutzt werden.*

Bildliche Darstellung des intakten Gehirns und pathophysiologische Mechanismen neurologischer und psychiatrischer Störungen

Störungen des Nervensystems sind ein drängendes gesellschaftliches Problem. Mehrere Gruppen des Fachbereichs Neurowissenschaften am MDC konzentrieren ihre Arbeit auf die Aufklärung molekularer Mechanismen der Entstehung von Krankheiten des Nervensystems mit dem Fernziel, gezielte Therapien mit z.B. niedermolekularen Wirkstoffen zu entwickeln.

Die Multiple Sklerose (MS) ist eine Autoimmunkrankheit, bei der die Immunreaktion des Patienten das zentrale Nervensystem angreift und die Myelinscheide der Axone zerstört. Befallene Nervenzellen verlieren ihre Fähigkeit zu kommunizieren. Die Gruppe von ***Frauke Zipp*** *studiert die molekularen Mechanismen, die MS verursachen und sucht nach den Molekülen, die am pathophysiologischen Prozess teilnehmen. Vor einiger Zeit konnte die Gruppe zeigen, dass das Kallikrein-Kinin-System an der Regulation von Entzündungen des ZNS beteiligt ist, indem es die enzephalitogene T-Lym-*

logical process. They recently showed that the kallikrein-kinin system is involved in the regulation of CNS inflammation, limiting encephalitogenic T lymphocyte infiltration into the CNS. By this, they identified a new molecular target, the kinin receptor B1, for the therapeutic treatment of chronic inflammatory diseases such as multiple sclerosis (Schulze-Toppenhoff et al., Nature Medicine 2009).

Gliomas comprise the majority of cerebral tumors, and patients diagnosed with glioma multiforme, a highly invasive glioma type, have a very poor prognosis. The group of **Helmut Kettenmann** found that microglial cells strongly promote glioma growth and invasion, and observed an interesting interplay between microglial and glioma cells. Glioma cells release the metalloprotease MMP2, which degrades the extracellular matrix and promotes invasion. This metalloprotease is, however, released in an inactive precursor form and requires processing for activation. The ectoenzyme MT1-MMPT does activate MMP2, but this enzyme is not produced by glioma cells. Instead, a factor released by the glioma cells activates toll-like receptors in the surrounding microglial cells, and upregulates microglial expression of MT1-MMP. Thus, glioma cells exploit microglial cells to promote their own invasion. Interfering with toll-like receptors or the signaling pathways used by these receptors might reduce the rapid expansion of glioma cells, and therefore microglia have become a new target for glioma research (Markovic et al., PNAS 2009, in press).

Many proteins of diverse sequence, structure and function self-assemble into fibrillar aggregates commonly termed amyloids. Amyloid formation is associated with protein-misfolding disorders, and plays important roles in neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease. The group of **Erich Wanker** explores if amyloid formation pathways can be redirected with the help of small molecules. They showed that EGCG, a compound originally isolated from green tea, potently inhibits the formation of huntingtin aggregates. In collaboration with the group of **Jan Bieschke**, they defined the mechanisms by which EGCG affects this process. The compound directly binds to the natively unfolded polypeptides and prevents their conversion into amyloid oligomers and protofibrils. Thus, this substance interferes with an early step in the amyloid formation cascade and redirects unfolded polypeptide molecules into an alternative aggregation pathway before they become amyloidogenic (Ehrnhoefer, Bieschke and others, Nature Struct Mol Biol. 2008).

phozyten-Infiltration begrenzt. Auf diese Weise konnte ein neues molekulares Angriffsziel, der Kinin Rezeptor B1, für die Behandlung chronischer inflammatorischer Prozesse wie der MS identifiziert werden (Schulze-Toppenhoff et al., Nature Medicine 2009).

*Die Mehrzahl der Hirntumoren sind Gliome. Speziell das Glioma multiforme ist eine sehr invasive Form, und davon betroffene Patienten haben eine sehr schlechte Überlebensprognose. Die Gruppe von **Helmut Kettenmann** hat festgestellt, dass Mikroglia-Zellen das Wachstum von Gliomen und ihre Invasionsneigung stark befördern und dass dabei eine Wechselwirkung zwischen Mikroglia und Gliomzellen besteht. Gliomzellen setzen die Metalloprotease MMP2 frei, die die extrazelluläre Matrix angreift und damit die Invasion ins Gewebe begünstigt. Dieses Enzym wird allerdings als inaktive Vorstufe freigesetzt und erst durch weitere Prozesse aktiviert. Das Ektoenzym MT1-MMPT aktiviert MMP2, wird in Gliomzellen jedoch nicht gebildet. Ein anderer Faktor aus den Gliomzellen hingegen aktiviert Toll-like Rezeptoren in benachbarten Mikrogliazellen, was in diesen zur Expression von MT1-MMP führt. Gliomzellen nutzen auf diese Weise Mikroglia als Helfer für die eigene Ausbreitung. Ein therapeutischer Eingriff auf Ebene der Toll-like Rezeptoren oder ihrer Signalkaskade könnte die rasche Ausbreitung der Tumorzellen verzögern. Mikroglia könnte somit ein neues Ziel der Gliomforschung werden (Markovic et al., PNAS, 2009, im Druck).*

*Zahlreiche Proteine verschiedenster Aminosäuresequenz, Raumstruktur und zellulärer Funktion lagern sich sich zu fibrillären Aggregaten zusammen, die üblicherweise Amyloide genannt werden. Amyloidbildung steht im Zusammenhang mit Erkrankungen, bei denen die Proteinfaltung gestört ist, und spielt bei neurodegenerativen Krankheiten wie Morbus Alzheimer, Morbus Parkinson oder Morbus Huntington eine wichtige Rolle. Die Arbeitsgruppe von **Erich Wanker** untersucht, ob die Amyloidbildung durch Einwirkung niedermolekularer Wirkstoffmoleküle beeinflußt werden kann. So konnte gezeigt werden, dass EGCG, eine Verbindung, die ursprünglich aus Grünem Tee isoliert worden war, die Bildung von Huntingtin-Aggregaten sehr wirksam hemmt. Zusammen mit der Gruppe von **Jan Bieschke** klärte die Wanker-Gruppe den Wirkungsmechanismus dieser Hemmung auf. Die Verbindung bindet direkt an noch ungefaltete Polypeptide und verhindert damit deren Umwandlung in Amyloid-Oligomere und Protofibrillen. Die Substanz greift also in einen frühen Schritt der Amyloid-Bildungskaskade ein und leitet den Prozess in einen anderen Aggregationsweg um, so dass er nicht amyloidbildend wirkt. (Ehrnhoefer, Bieschke and others, Nature Struct Mol Biol. 2008).*

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Developmental Biology/ Signal Transduction

We analyze the functions of signaling molecules and of transcription factors in development of the nervous system and muscle. For this work, we use mice as a model organism. The molecular genetics of mice is well developed, and homologous recombination combined with embryonic stem cell technology can be used to introduce deletions or insertions into the genome. A further development of the technique, the Cre/LoxP technology, allows us now to introduce conditional mutations that are restricted to a particular cell lineage. We have used these technologies to analyze genes that control myoblast fusion, and showed that two small G proteins, Rac1 and Cdc42, are essential for the fusion process. In addition, we identified the function of several transcription factors in development of the nervous system. Among these is a novel factor, Insm1, that we found unexpectedly to perform also important functions in development of pancreatic beta-cells, the insulin-producing endocrine cells.

The role of Insm1 in neuronal development

Robert Storm, Jochen Welcker, Kira Balueva and Shiqi Jia (in collaboration with John Jacob and James Briscoe, MRC, London).

Insm1 (insulinoma associated antigen) encodes a Zn-finger factor that is transiently expressed in differentiating neurons throughout the developing nervous system, as well as in endocrine cells of the pancreas and intestine. We generated mice with a targeted mutation to analyze the function of the Insm1 gene. In an initial analysis, we identified Insm1 as a factor crucial for the differentiation of beta-cells in the pancreas. Impaired function or loss of pancreatic beta-cells causes diabetes, a prevalent humane disease throughout the world. In the absence of Insm1, the expression program for hormones and a plethora of genes coding for proteins involved in secretion and vesicle transport was downregulated in beta-cells.

However, Insm1 is not only required in endocrine cell development, but is also a crucial component of the transcriptional network that controls neuronal development. In Insm1 mutant mice, differentiation of sympatho-adrenal precursors was strongly delayed, which was accompanied by reduced proliferation of the precursors. Whereas sympathetic neurons differentiated late and in reduced numbers, resulting in small sympathetic ganglia, terminal differentiation of adrenal chromaffin cells, the major source of noradrenaline, did not occur. The catecholamine noradrenaline is essential for fetal heart function and survival. Due to a pronounced noradrenaline deficiency, Insm1 homozygous mutant mice died during mid-gestation, but we could rescue them by administration of catecholamine intermediates. Analysis of the transcriptional network governing sympatho-adrenal precursor differentiation indicated that Insm1 acts downstream of Mash1 (Ascl1) and Phox2b.

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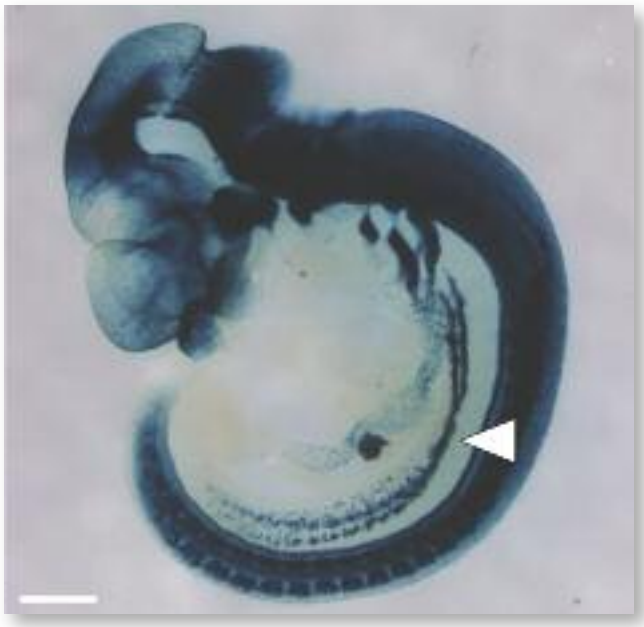


Figure 1. *Insm1 expression in the developing embryo. To analyze Insm1 expression, we took advantage of the Insm1lacZ allele in which lacZ sequences replace the Insm1-coding sequence. Insm1lacZ/+ mice. LacZ expression is detected in the entire primary sympathetic ganglion chain (arrowhead), as well as in sensory ganglia and in the central nervous system.*

In a collaborative effort, we could also show that Insm1 controls the differentiation of monoaminergic neurons in the hindbrain. These neurons use either the neurotransmitter serotonin or noradrenaline, both of which have a wide range of complementary actions and are implicated in the pathophysiology of many common neurological and psychiatric disorders. In Insm1 mutant mice, serotonergic precursors located in the ventral hindbrain, began to differentiate despite the reduced expression of the postmitotic serotonergic fate determinants (Pet1, Lmx1 and Gata2), but failed to produce serotonin due to the lack of tryptophan hydroxylase 2, an enzyme essential for serotonin biosynthesis. In brainstem noradrenergic centers of Insm1 mutants, expression of tyrosine hydroxylase was impaired, resulting in aberrant noradrenergic differentiation.

The bHLH factor Olig3 functions in the determination of neuronal fates

Justyna Cholewa-Waclaw, Robert Storm, Dominique Bröhl, Thomas Müller (in collboration with Mathias Treier, EMBL Heidelberg)

The hindbrain is the part of the central nervous system that monitors and regulates inner organ function such as the rate of heartbeat, blood pressure and breathing. To achieve this, hindbrain neurons receive and integrate viscerosensory information from inner organs. The complex circuitry in which neurons participate is established during development and depends on their spatially and temporally ordered appearance.

One important class of genes that regulates cellular diversity in the nervous system encodes basic helix-loop-helix (bHLH) transcription factors. The Olig3 bHLH factor is expressed in the ventricular zone of the dorsal

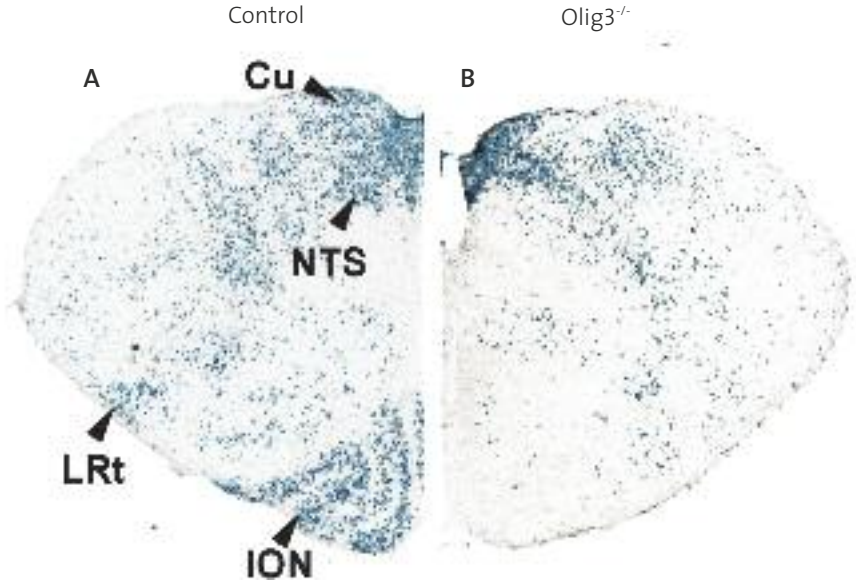


Figure 2. ***Genetic lineage tracing in heterozygous and homozygous Olig3 mutant mice.*** *Cre expression that is driven by the Olig3 locus induces recombination in the Rosa26R allele, allowing expression of a lacZ gene. The active, recombined lacZ gene is inherited in cells that expressed have expressed Olig3 (and therefore the cre recombinase) at earlier stages in development. Cells that express lacZ were identified by X-Gal staining (blue). Shown is the medulla oblongata of control (A: Olig3CreERT2/+; Rosa26R) and Olig3 mutant (B: Olig3CreERT2/-; Rosa26R) mice at E18.5. Arrowheads indicate cuneate nucleus (Cu), inferior olivary nucleus (ION), nucleus of the solitary tract (NTS) and lateral reticular nucleus (LRt). Note the absence of inferior olivary nucleus and nucleus of the solitary tract, as well as the changed distribution of neurons in the dorsal hindbrain of the homozygous Olig3 mutant mice.*

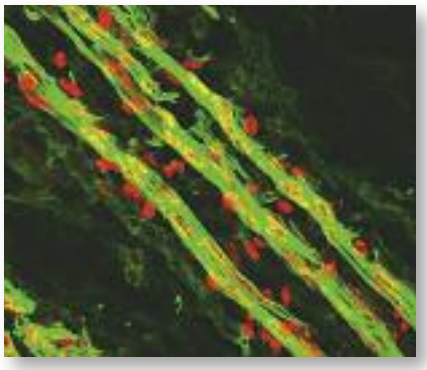
alar plate of the hindbrain. We found that the Olig3+ progenitor domain gives rise to several neuronal subtypes in the dorsal alar plate. We used genetic lineage tracing to demonstrate that these neurons settle in the nucleus of the solitary tract and to precerebellar nuclei. The fate of class these neurons is not correctly determined in Olig3 mutant mice, and as a consequence, the nucleus of the solitary tract does not form, and precerebellar nuclei, such as the inferior olivary nucleus, are absent or small.

The small G-proteins Rac1 and Cdc42 are essential for myoblast fusion in the mouse

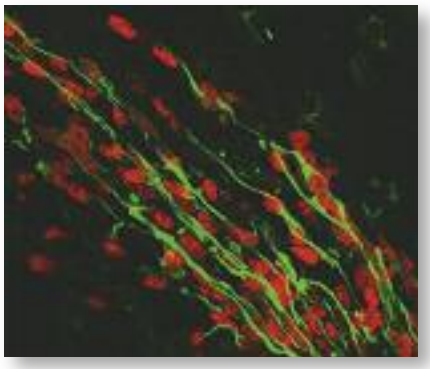
Elena Vasyutina, Benedetta Martarelli, Hagen Wende (in collaboration with Cord Brakebusch, University of Copenhagen)

Skeletal muscle fibers are syncytia that arise by the fusion of myogenic cells. Mononucleated myogenic cells, the myoblasts, fuse with each other to form multinucleated myotubes. During development and in the adult, myoblast fusion allows generation, growth and repair of muscle fibers. Rac1 and Cdc42 are small G-proteins that regulate actin dynamics. In Drosophila, Rac GTPases, dRac1 and dRac2, act downstream of cell adhesion molecules to control cytoskeletal rearrangements, and myoblast fusion.

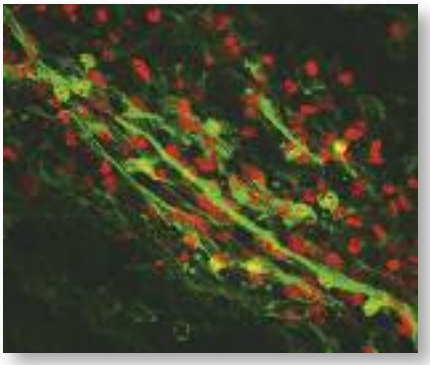
We analyzed the function of Rac1 and Cdc42 in myogenesis using conditional mutagenesis in mice. We showed that in the absence of Rac1 and Cdc42, myoblast fusion is severely compromised in vivo and in vitro. The deficit in fusion of Rac1 or Cdc42 mutant myoblasts correlated with a deficit in the recruitment of actin fibers and vinculin to myoblast contact sites. Moreover, we demonstrated that Rac1 and Cdc42 are required in both fusion partners. Thus, our analysis demonstrated that the function of Rac1 is evolutionarily conserved from insects to mammals, and that Cdc42, a molecule hitherto not implicated in myoblast fusion, is essential for the fusion of murine myoblasts.



Control



$Rac1^{fl/fl}$;$Lbx1^{cre}$



$Cdc42^{fl/fl}$;$Lbx1^{cre}$

Figure 3. Myoblast fusion in mice requires Rac1 and Cdc42. *In the muscle of a control animal (A), single myoblasts (visualized by MyoD in red) fuse to generate multinucleated myotube. In conditional Rac1 (B) or Cdc42 (C) mutant muscle, myoblasts do not fuse and myofibers remain short and thin.*

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Vasyutina E, Martarelli B, Brakebusch C, Wende H, Birchmeier C (2009) The small g-proteins rac1 and cdc42 are essential for myoblast fusion in the mouse. Proc Natl Acad Sci U S A 106: 8935-8940

Storm R, Cholewa-Waclaw J, Reuter K, Brohl D, Sieber M, Treier M, Muller T, Birchmeier C (2009) The bhlh transcription factor olig3 marks the dorsal neuroepithelium of the hindbrain and is essential for the development of brainstem nuclei. Development 136: 295-305

Wildner H, Gierl MS, Strehle M, Pla P, Birchmeier C (2008) Insm1 (ia-1) is a crucial component of the transcriptional network that controls differentiation of the sympatho-adrenal lineage. Development 135: 473-481

Hori K, Cholewa-Waclaw J, Nakada Y, Glasgow SM, Masui T, Henke RM, Wildner H, Martarelli B, Beres TM, Epstein JA, Magnuson MA, Macdonald RJ, Birchmeier C, Johnson JE (2008) A nonclassical bhlh rbpj transcription factor complex is required for specification of gabaergic neurons independent of notch signaling. Genes Dev 22: 166-178

Gierl MS, Karoulias N, Wende H, Strehle M, Birchmeier C (2006) The zinc-finger factor insm1 (ia-1) is essential for the development of pancreatic beta cells and intestinal endocrine cells. Genes Dev 20: 2465-2478

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Physiology and Pathology of Ion Transport

Ion transport across cellular membranes is important for cellular homeostasis and has integrative functions such as transepithelial transport or neuronal signal transduction. We study these processes at various levels, from biophysical analysis of transport proteins, structure-function analysis, role in cellular functions such as cell volume regulation or endocytosis, to the role in the organism. The physiological role of ion transport proteins has often been gleaned from pathologies resulting from their inactivation in human diseases or in mouse models. We have discovered several human 'channelopathies' and have generated and analyzed many mouse models.

We focus on CLC chloride channels and transporters, KCC potassium-chloride co-transporters and KCNQ potassium channels, and are currently extending our interest to other channel classes. The mutational inactivation of these ion transport proteins led to pathologies ranging from epilepsy, deafness, lysosomal storage disease to osteopetrosis, kidney stones and hypertension. We are particularly interested in the control of neuronal excitability and in the role of chloride and pH in endosomes and lysosomes.

CLC chloride channels and transporters

The CLC gene family, discovered in our laboratory in 1990, encodes plasma membrane chloride channels and chloride transporters of intracellular membranes. In the past couple of years, we identified associated β-subunits, discovered that certain vesicular CLCs are electrogenic Cl^-/H^+-exchangers, performed structure-function analysis, and uncovered several new pathologies resulting from their dysfunction.

An inner ear-specific knock-out of the Cl^- channel β-subunit barttin explains deafness in human Bartter syndrome type IV

Gesa Rickheit, Hannes Maier, Anselm Zdebik

Human Bartter syndrome type IV is associated with severe renal loss of fluid and salt, as well as with congenital deafness. We have previously shown that barttin, the protein encoded by the *BSND* gene that is mutated in that disease, is an accessory β-subunit of the chloride channels ClC-Ka and -Kb. Both channels are expressed in the kidney and in the epithelium of the stria vascularis of the cochlea. We now generated a conditional 'floxed' barttin KO mouse to delete barttin specifically in the inner ear, thereby avoiding the massive salt and fluid loss that leads to early postnatal death. Like patients, these mice are congenitally deaf. We showed that this is due to a collapse of the positive voltage in the scala media (the endocochlear potential) which is necessary to drive a depolarizing influx of potassium through mechanosensitive channels in sensory hair cells. Outer hair cells were no longer able to mechanically amplify sound in the inner ear and eventually degenerated. We have thus clarified the pathological mechanism underlying a form of human deafness and have revealed a previously unknown role of chloride channels in the generation of the endocochlear potential.

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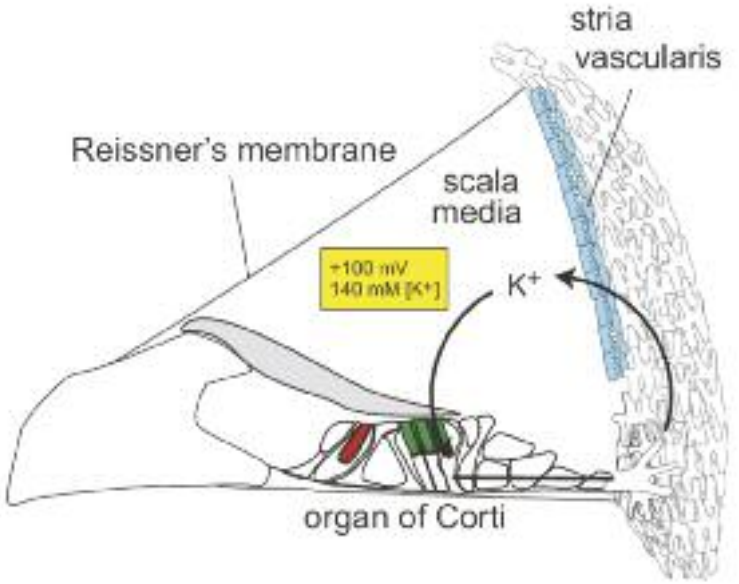

Figure 1. ***Model for potassium recycling in the inner ear.*** *The fluid space of the scala media is in direct contact with the apical membranes of inner hair cells (shown in green) and outer hair cells (red). Its unusually high K^+ concentration and positive voltage (+100 mV), both generated by the epithelium of the stria vascularis, are needed to drive a depolarizing K^+ current through mechanosensitive channels into those sensory hair cells. Potassium then leaves hair cells at their basal pole and is transported back to the stria.*

ClC-7 is needed for normal protein degradation in renal proximal tubules

Lena Wartosch, Jens Fuhrmann, Tobias Stauber

ClC-7 is a lysosomal Cl^-/H^+ exchanger broadly distributed across tissues. We have previously shown that its disruption leads to osteopetrosis and lysosomal storage disease in mice and men. We now generated a conditional ClC-7 KO that allowed us, on the one hand, to follow the CNS pathology much further and to show that it is owed to a cell-intrinsic effect on neurons. To investigate the lysosomal storage disease that is observed in the kidney, we selectively inactivated ClC-7 in proximal tubules. Combined endocytosis/protein degradation experiments *in vivo* showed for the first time that the lack of ClC-7 significantly slows protein degradation. The enlargement of lysosomal-like compartments in KO proximal tubules, however, is not due to excessive protein accumulation because it could not be prevented by the simultaneous KO of ClC-5 that is crucial for endocytotic uptake.

Role of the vesicular chloride transporter ClC-3 in neuroendocrine cells

Tanja Maritzen, Damien Keating, Ioana Neagoe, Anselm Zdebik

ClC-3 is a Cl^-/H^+-exchanger that is expressed on endosomes and synaptic vesicles. We have previously shown that its KO leads to a severe neurodegeneration which results in a complete absence of the hippocampus after a few months. However, ClC-3 is broadly expressed in many tissues. Newly generated antibodies allowed us for the first time to investigate its tissue expression by immunocytochemistry. We found that ClC-3 is highly expressed in several neuroendocrine tissues, including adrenal glands and pancreatic islets, where it is expressed in all secretory cell types. ClC-3 was detected in endosomes in synaptic-like microvesicles, but not in large dense core vesicles that are responsible e.g. for insulin or adrenalin secretion in β-cells or chromaffin cells, respectively. Nonetheless, stimulated secretion of either hormone was reduced in ClC-3 KO cells, suggesting an indirect effect of ClC-3 on large dense core vesicle exocytosis.

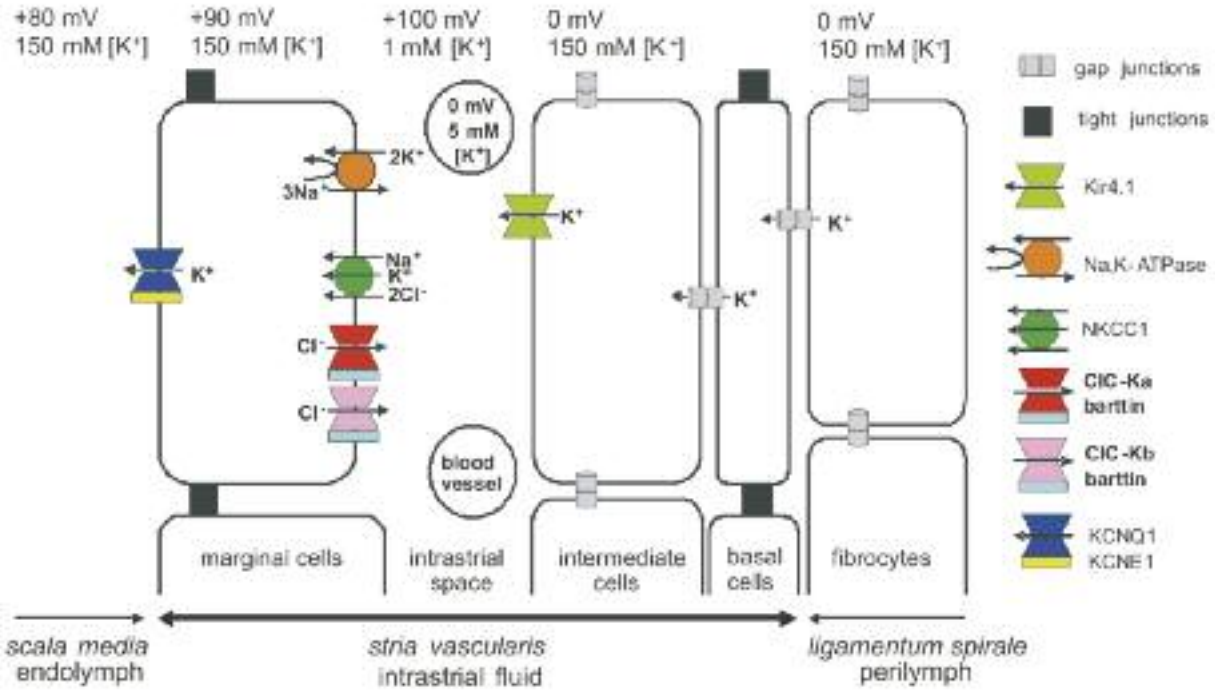

Figure 2. ***Cellular model for ionic transport across the stria vascularis.*** *In this multilayered epithelium, potassium is secreted across the apical membrane of marginal cells through KCNQ1/KCNE1 K^+-channels. Potassium is taken up basolaterally through the combined transport activity of the Na,K-ATPase and the NaK2Cl cotransporter NKCC1. The latter transporter needs Cl^- channels for recyling. Our work has shown that this task is carried out by ClC-Ka/barttin and ClC-Kb/barttin Cl^- channels. If the β-subunit is missing as in patients with Bartter syndrome type IV or in our KO mouse model, potassium secretion is impaired and the K^+-concentration in the extracellular space between marginal and intermediate cells increases. This leads to a dramatic drop of the endocochlear potential, which is largely generated by a K^+-diffusion potential through Kir4.1 K^+-channels in the apical membrane in intermediate cells. This drop in potential leads to deafness.*

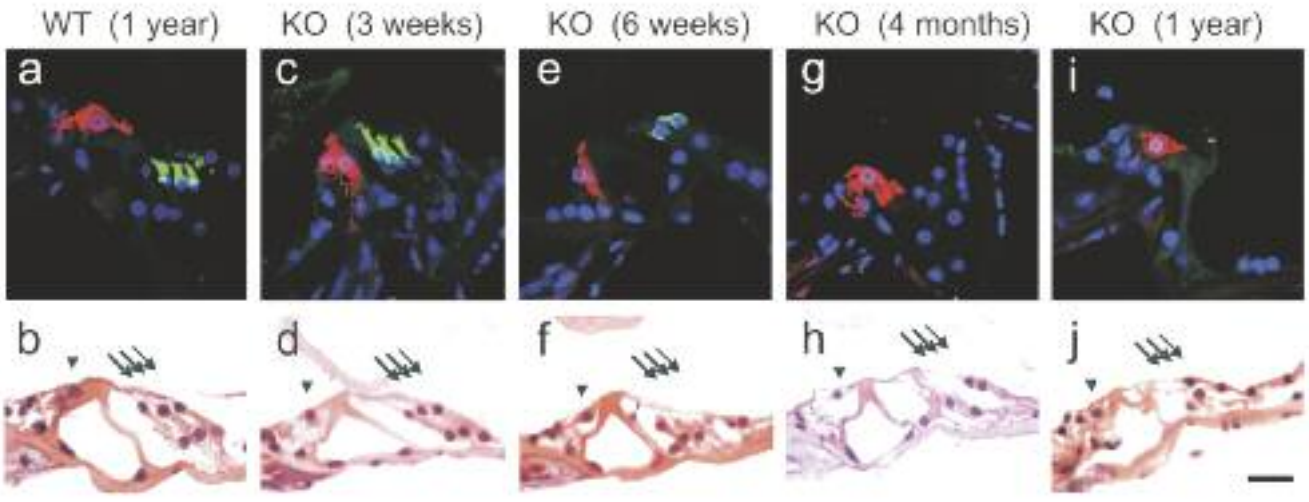

Figure 3. ***Selective degeneration of outer hair cells in barttin KO mice.*** *The drop in endocochlear potential owed to a loss of barttin leads to a functional impairment of outer hair cells and later also to their degeneration, as shown by immunocytochemistry (upper row; inner hair cells stained in red, outer hair cells in green) and hematoxin-eosin staining (below, outer hair cells indicated by arrows).*

Determinants of anion-proton coupling in mammalian endosomal CLCs

Anselm Zdebik, Eun-Yeong Bergsdorf, Michael Pusch, Giovanni Zifarelli

Similar to the bacterial EcClC-1 protein, and in contrast to their previous classification as Cl^--channels, ClC-4 and ClC-5 are antiporters that exchange chloride for protons. By mutagenesis and biophysical analysis, we showed that a glutamate at the cytoplasmic face of the transporter is important for proton coupling. It apparently serves as a proton acceptor. Cl^-/H^+-transport persists when this residue is mutated to other titratable amino-acids, but transport is altogether stopped when it is mutated e.g. to alanine. Transport is restored when a centrally located 'gating glutamate' is neutralized, which eliminates Cl/proton coupling at a central exchange site and therefore eliminates the need of protons for Cl transport.

Residues important for nitrate transport in plant and mammalian CLC proteins

Eun-Yeong Bergsdorf, Anselm Zdebik

AtClC-a from the plant Arabidopsis has been shown by Barbier-Brygoo and colleagues to function as a NO_3^-/H^+ exchanger with which plants accumulate the nutrient nitrate into their vacuoles. This requires tightly coupled countertransport. However, when nitrate is transported by mammalian endosomal ClC-4 and ClC-5, it is largely uncoupled from protons. Sequence comparison identified a serine in a highly conserved signature sequence of ClC-4/5 that is exchanged by a proline in AtClC-a. Mutating the serine of ClC-5 to proline led to tightly coupled nitrate/proton exchange, whereas the plant transporter lost its preference for nitrate when its proline was mutated to serine. The ClC-0 Cl channel from electric fish also gained nitrate selectivity when this proline was introduced. We have thus identified a residue crucial for anion selectivity and have also been able for the first time to functionally express a plant CLC in animal cells.

KCl and NaK2Cl cotransporters

Carsten Pfeffer, Guillermo Spitzmaul, Patricia Seja, Valentin Stein, Hannes Maier

We have previously knocked-out all KCl-cotransporter isoforms (KCC1-4) in mice which led to 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. We are currently investigating various mouse lines in which KCC2 has been inactivated in specific sets of neurons.

We have also studied the transporter that is the major player in elevating cytoplasmic chloride in neurons before the expression of KCC2 kicks in, namely the NaK2Cl cotransporter NKCC1. We found that NKCC1 is an important, though not the only, transporter elevating intraneuronal chloride. The excitability and spontaneous network activity of hippocampal slices were reduced in the first 10 days after birth. This correlated with a delay in synapse maturation. However, in contrast to speculations by others, no morphological changes were observed.

KCNQ potassium channels

Matthias Heidenreich, Guillermo Spitzmaul, Vitya Verdanyan, Pawel Fidzinski

There are five different isoforms of KCNQ (Kv7) potassium channels, KCNQ1- KCNQ5. KCNQ2-KCNQ5 mediate 'M-currents' that regulate neuronal excitability. We had previously 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. We are now investigating possible vestibular phenotypes in these mice and are generating other mouse models.

Selected Publications

Blanz J., Schweizer M., Auberson M., Maier H., Muenscher A., Hübner C.A., Jentsch T.J. (2007). Leukoencephalopathy upon disruption of the chloride channel ClC-2. J. Neurosci. 27, 6581-6589.

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Bergsdorf, E.-Y., Zdebik A.A., Jentsch T.J. (2009). Residues important for nitrate/proton coupling in plant and mammalian CLC transporters. J. Biol. Chem. 284, 11184-11193.

Fritz G. Rathjen

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Neuronal Connectivity

The functioning of the nervous system is critically dependent on the correct wiring of neurons which is primarily established during embryonic and early postnatal development. To generate precise circuits neuron extends an axon and dendrites which are guided to their targets by growth cones. This highly motile structure at the tip of axons or dendrites is steered by molecular guidance cues located in the local environment. In the target area neurons then establish synapses that are the fabric of communication between neurons. Our research group focuses currently on the following molecular aspects on the formation of neuronal connectivity: branching of axons and dendrites and formation and maturation of synapses.

Axonal and dendritic branching are key steps in regulating neuronal connectivity

One important prerequisite to generate the complex circuits of the mature nervous system is the arborization of axons and dendrites. This key process enables an individual neuron to innervate multiple targets whereby information from neurons in various locations can be integrated. Branching is therefore a critical step in the formation of complex neuronal circuits. Despite its importance the extracellular signals and the intracellular signaling machinery that regulate branching have remained poorly understood. To investigate these processes we concentrated on sensory axons projecting into the spinal cord and on cortical neurons.

A cGMP signaling cascade is essential for branching of sensory axons

Sensory axons enter the spinal cord at the dorsal root entry zone where they branch into a rostral and a caudal arm. These two arms remain confined to lateral regions of the cord and grow over several segments in both directions. Collaterals are then generated by budding from these stem axons which extend into the gray matter of the cord. Sensory axons therefore reveal at least two branching modes: splitting of the growth cone when arriving at the cord followed by budding of collaterals (interstitial branching) from the stem axons.

By genetic ablation and by single axon tracing we revealed that a cGMP-dependent signaling cascade is important for sensory axon branching at the dorsal root entry zone. In the absence of either the natriuretic peptide C (CNP), the receptor guanylyl cyclase Npr2 or the cGMP-dependent kinase I (cGKI) axons are unable to form a T-shaped branch when entering the spinal cord (Figure 1 and Figure 2). Instead axons turn in rostral or caudal directions. The other branching mode – the formation of collaterals – is not regulated by this signaling cascade.

Our current efforts concentrate on the identification of further upstream and downstream components to establish a complete picture of this signaling cascade as well as on other axon systems that regulate branching by this signaling cascade.

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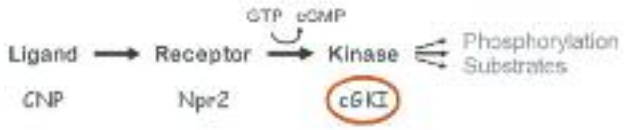

Figure 1. ***A cGMP signaling cascade important for axonal bifurcation.***

Our starting point was the cGKI (encircled) which we showed to be important for branching. During the past funding period we used genetic ablation techniques to characterize the receptor guanylyl cyclase Npr2 and its ligand CNP (natriuretic peptide C) and showed that these components are essential for bifurcation. Furthermore, several phosphorylation targets have been identified by biochemical approaches; however, it is currently unknown whether they are also important for bifurcation.

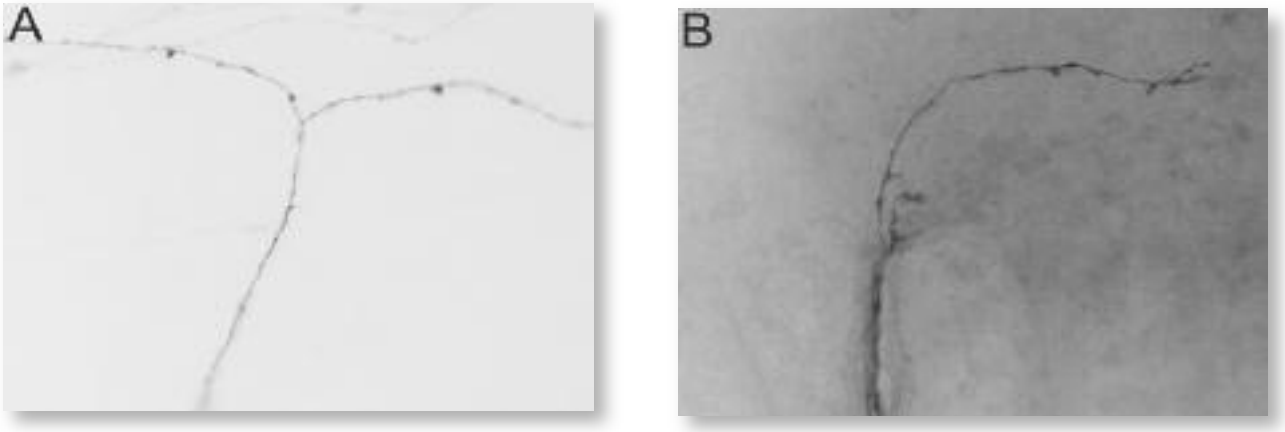

Figure 2. ***Impairment of bifurcation in the absence of cGMP signaling.***

A shows a single bifurcating sensory axon in a normal mouse embryo while B reveals an axon in the absence of CNP signaling which turns but does form T-shaped branches.

The transmembrane protein CALEB is implicated in synapse maturation

By a detailed biochemical and histological characterization we revealed that CALEB is primarily localized in the somato-dendritic compartment of a neuron and partially co-localizes with synapses (Figure 3). It is strongly expressed in early postnatal stages of the mouse when the majority of synapses and dendritic trees are formed.

Dendrites are the input regions of a neuron. In many cases they arborize into a tree like structure by an extensive and complex branching program. Early stages of dendrite development might be regulated by intrinsic programs specific for a neuron type which might then be modulated by different external signals detected by specific receptor proteins. During the development of dendritic trees CALEB appears to be required for specific aspects of synapse maturation. For example, CALEB-deficient synapses displayed higher

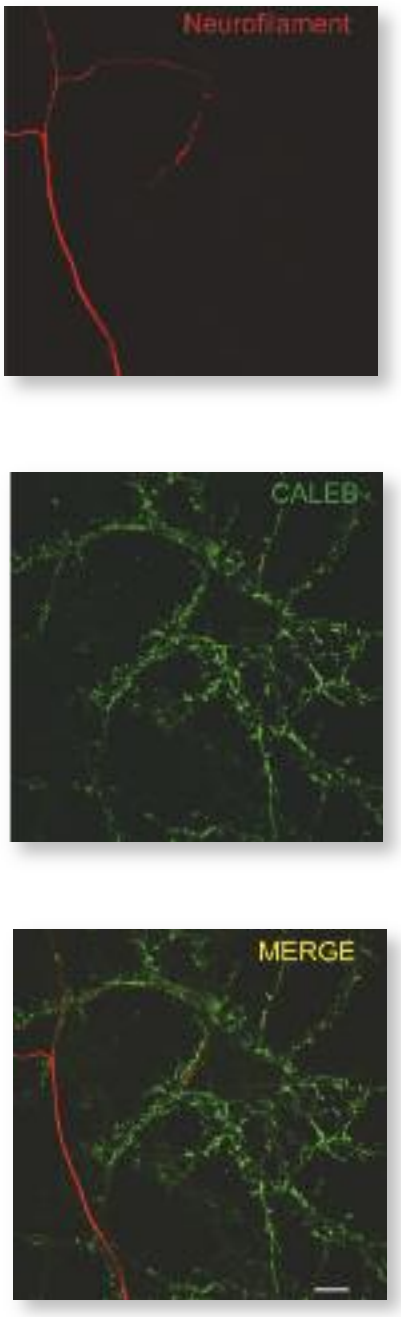

Figure 3. ***Localization of CALEB on dendrites.***

CALEB (green) is primarily found on structures that are positive for MAP2 – a marker for dendrites – and appears to be absent from axons which are stained by anti-neurofilament (red).

paired-pulse facilitation, less depression during prolonged repetitive activation and a lower rate of spontaneous postsynaptic currents that are based on a lower release probability of neurotransmitters at early but interestingly not at mature postnatal stages.

CALEB is a transmembrane protein composed of an N-terminal segment that contains chondroitinsulfate chains followed by an acidic stretch, and an EGF-like domain, a transmembrane and a cytoplasmic segment. The EGF-like domain of CALEB is related to the EGF domains of the neuregulins, TGFα or EGF itself and therefore CALEB might be considered to be a member of the EGF-family of differentiation factors. CALEB appears to be generated as a precursor protein that becomes converted in a truncated transmembrane form with an exposed EGF domain. In slices and in cultures this conversion occurs at the cell surface which is facilitated by membrane depolarization and calcium influx through voltage gated calcium channels.

The understanding of the cell biological function of CALEB in the development of neuronal circuits is of great interest since genetic linkage studies identified CALEB as a putative susceptibility gene for schizophrenia in humans which is considered as a disorder of development.

Neural cell adhesion proteins and their function in the formation of neural networks

Cell adhesion proteins of the Ig superfamily, the cadherins or the neurexins are thought be essential for the formation of neuronal circuits. Currently we are characterizing the Ig superfamily member CAR (coxsackie-adenovirus receptor) that is strongly expressed at developmental stages and then becomes down regulated in the mature brain. CAR is a transmembrane protein composed of two Ig-like domains and an intracellular segment that becomes alternatively spliced. Our electrophysiological recordings and calcium imaging measurements using wildtype and knockout neurons reveal that CAR is not simply an adhesion protein. In contrast, manipulation of CAR by using a specific ligand indicates that CAR affects intracellular calcium signaling and thereby influences the development of neuronal networks. Binding as well as structural studies demonstrate homophilic and heterophilic interactions of CAR for which specific extracellular domains are important.

Selected Publications

Schmidt, H., Stonkute, A., Jüttner, R., Koesling, D., Friebe, A., F.G. and Rathjen, F.G. (2009) C-type natriuretic peptide (CNP) is a bifurcation factor for sensory neurons, PNAS, 106, 16847-16854.

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Jüttner, R., Moré, M.I., Das, D., Babich, A., Meier, J., Henning, M., Erdmann, B., Müller, E.C., Otto, A., Grantyn, R., and Rathjen, F.G. (2005). Impaired synapse function during postnatal development in the absence of CALEB, an EGF-like protein processed by neuronal activity. Neuron, 46, 233-245.

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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 the lab.

Molecular Basis of Mechanotransduction

Yinth Andrea Bernal-Sierra, Liudmilla Lapatsina, Stefan Lechner, Alexey Kozlenkov

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. 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 mechanotransducing channel. We have cloned new vertebrates 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 SLP3 (stomatin like protein-3) was cloned by our group, and we subsequently generated a mouse model with a null mutation of the SLP3 locus. In SLP3 mutant mice many mechanoreceptors (or touch receptors) in the skin do not work in the absence of the SLP3 protein. In order to analyze touch sensation in mice we also developed a novel behavioral assay 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. We were very pleased to find that SLP3 mutant mice have severe deficits in their ability to detect such textured surfaces. Current work in the lab focuses on the role of related members of the stomatin-domain family in mechanotransduction, structure function studies and the identification of further essential interaction partners for SLP3.

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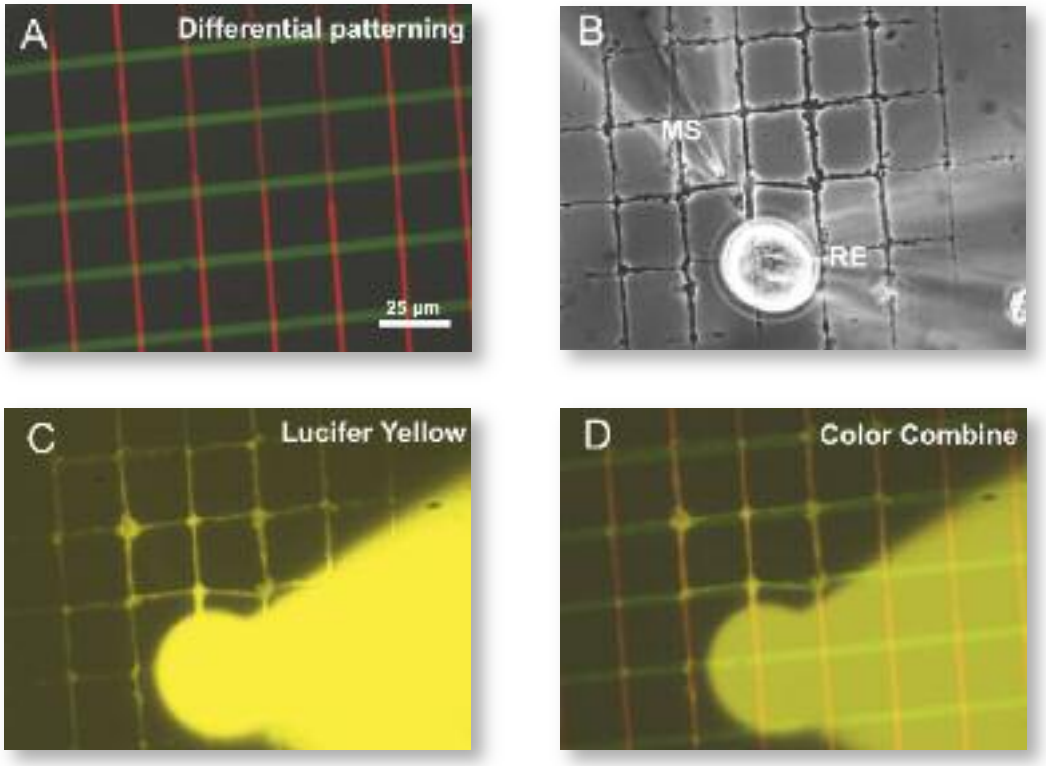
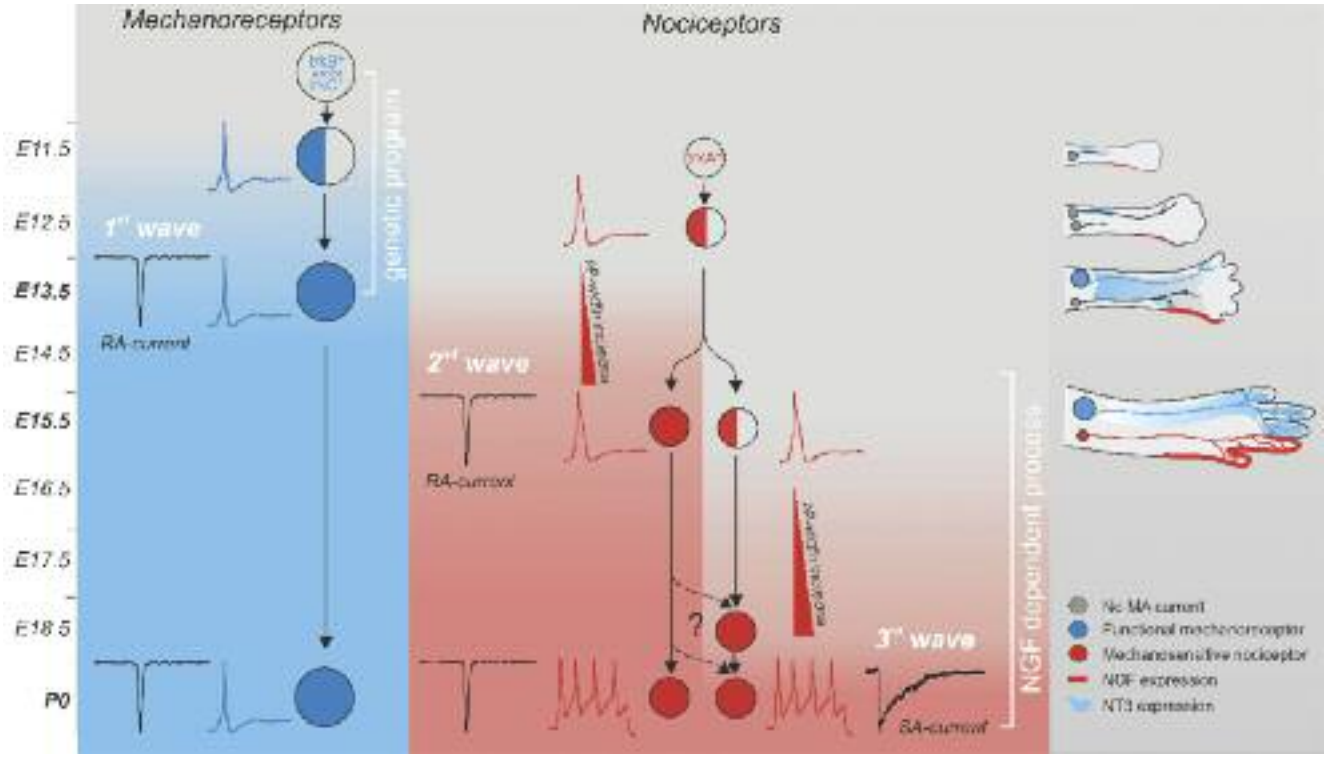


A single sensory neuron growing on a check patterned substrate. Top right (A) shows the stripes of laminin printed onto a glass surface. Note that different proteins can be printed in the vertical (red) or horizontal axis (green). (B) Phase contrast picture of a sensory neuron growing on the patterned substrate. Note that the neuron only grows neurites along the stripes to form a window like pattern of growth. In this picture the neuron was recorded with a patch pipette (RE) and the neurites can be mechanically stimulated with a nanomotor (MS). (C) The neurons was filled with a yellow dye via the recording pipette to show that neurites belong to the indicated cell. (D) Colour combine showing the pattern together with the yellow neurons and its neurites.

Neuronal nanodetection and micro-patterning, engineering sensory neurons for function

Li-Yang Chiang, Jing Hu, and Kate Poole

The mechanosensitive ion channels that are expressed by sensory 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. 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



Model summarizing the three waves of mechanosensitivity acquisition in different sensory neuron subtypes. Developmental stage is depicted from top to bottom for mechanoreceptors (blue) and nociceptors (red). Cartoons (right) depict the innervation of the limb with the known distribution of the neurotrophins NT-3 and NGF. Figure reproduced from Lechner et al. 2009 EMBO J.

sensory neurons can be made to grow to produce highly structured patterns in vivo (see Figure 1). Another application 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.

Touch, hearing and the development of mechanosensation

Henning Frenzel, Regina Hartl, Stefan Lechner, Nevena Milenkovic, Simone Pifferi

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 are measuring pyschometric functions in normals and hearing impaired people in order to describe quantitatively differences in the perception of touch. We are also carrying out a large twin study, to examine the heritability of touch acuity in humans. Initial results suggest that genetic factors are very important in determining how good our sense of touch is. We are also pursuing the hypothesis that some of the genetic factors influencing touch may also directly affect the second mechanosensory sense, hearing.

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.

Tuning pain sensitivity

Stefan Lechner, Nevena Milenkovic, Rui Wang

Nociception is describes our ability to respond to potentially or actually damaging stimuli. An important aspect of the biology of nociception is that after injury people and animals become much more sensitive to sensory stimulation than before injury. This phenomenon is sometimes called sensitization and it is often desirable to block this process after inflammation to prevent pain becoming pathologically severe. We are interested in the cellular and molecular basis of sensitization. We recently discovered that some endogenous chemicals, such as ATP and UTP that are released from damaged cells during inflammation can potently increase the magnitude of the mechanosensitive current in sensory neurons. This would have the effect of making nociceptors innervating inflamed tissue more sensitive to mechanical stimuli, and such a phenomenon may underlie the tenderness that follows inflammation. Identification of the mechanotransducer as a target of inflammation indicates that, this as yet unknown ion channel, may be an excellent molecular target to block in order to treat pain after inflammation.

Sensitization also happens to heat stimuli, a familiar example is the increased sensitivity to even moderate temperature that we experience following UV induced-sunburn. We recently identified the ligand of the tryosine kinase receptor c-Kit as a very potent factor in increasing the sensitivity of nociceptors to noxious heat. There are already clinically available potent blockers of c-Kit e.g. Gleevec, that are used to treat a variety of cancers. We are present carrying out a patient based study to determine if Gleevec and related compounds have significant analgesic effects when used in man. This study is being carried out within the ECRC at the MDC and at the Charité university hospital.

The Naked Mole Rat a pain free mammal?

Damir Omerbasic, Ewan St. John Smith

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). Interestingly, although this animal has normal acute pain responses it displays no hypersensitivity (so called hyperalgesia) to a variety of inflammatory and chemical stimuli. What is particularly striking in the naked mole rat is that the animals completely lack a neuronal or behavioral response to acid. We suspect that at the heart of this specialized adaptation lies in distinct gene variants encoding ion channels and associated channels that are required for the transduction of painful stimuli. We are at present cloning and characterizing genes coding ion channels from the naked mole rat to address this issue. We have cloned and started to characterize the naked mole rat capsaicin receptor, an ion channel called TRPV1 as well as the tyrosine kinase receptor trkA the activation of which by NGF can potently potentiate TRPV1. We are presently embarking on a large scale molecular characterization of the naked mole rat transcriptome using so-called deep sequencing technologies (In collaboration with Wei Chen, BIMSB).

Selected Publications

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Ines Ibañez-Tallon

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Molecular Neurobiology of Cell-surface Channels and Receptors

Ion channels and neurotransmitter receptors control cellular communication between nerve cells influencing neuronal excitability and synaptic transmission. Our group is interested in dissecting the contribution of individual channels and receptors on neuronal function in the mammalian nervous system. We use a combination of molecular, behavioral, electrophysiological, and genetic tools for the functional analysis of ion channels in the development of the nervous system and in disease, using mice as a model organism.

Genetic control of neural circuits

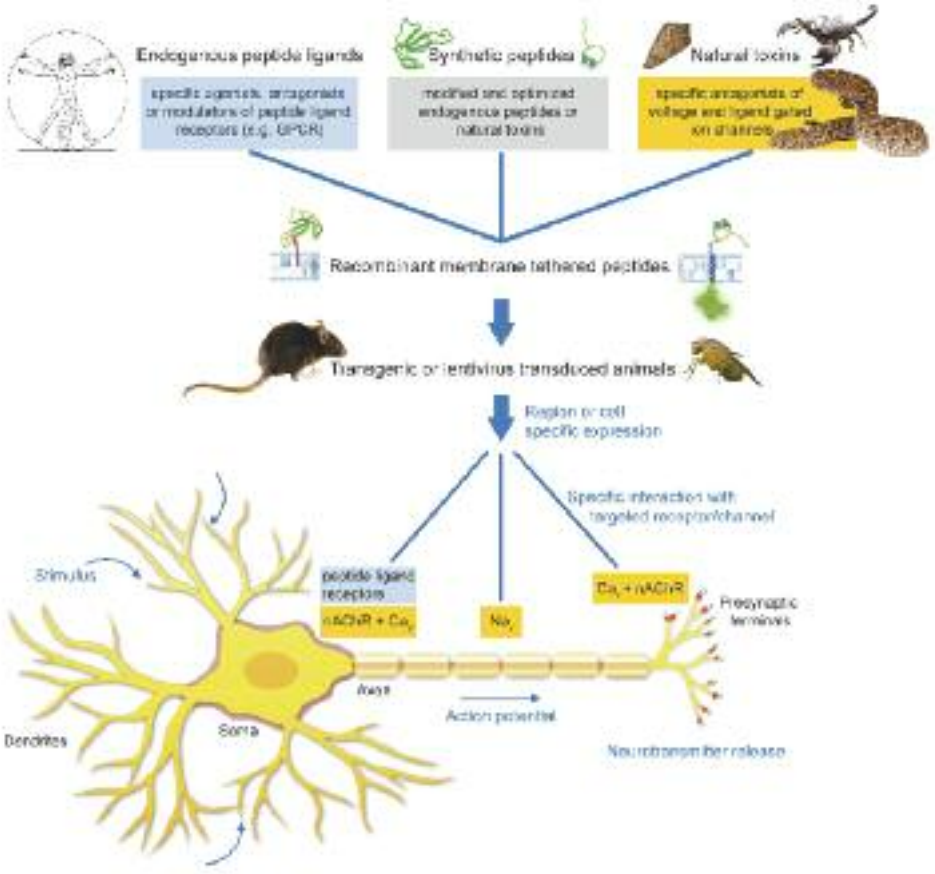
Contemporary genetic methods allow cell-selective targeting within complex neural circuits. We have recently developed a novel genetic method to manipulate ionic currents in vivo using genetically encoded cell-surface anchored toxins and neuropeptides, based on the discovery of endogenous prototoxins. The ongoing projects in our group are aimed at silencing or manipulating specific ion channels in defined neuronal circuits. The questions we are addressing are: how a particular class of ion channels in one cell population contributes to the function of a given neuronal circuit, and whether silencing one cell population has an impact in only that circuit and/or also affects the next circuit. Another question of interest is whether these functions can be restored upon reversibly inhibiting the expression of the cell-surface toxin or peptide. To approach these questions, we are focusing on specific neuronal circuits in which manipulation of certain ion channels could help dissecting the cascade of events that leads to chronic pain, hearing impairment, and nicotine mediated effects. To target these circuits, we are using genetic approaches such as BAC and knock-in transgenesis and lentiviral vectors to achieve cell-specific and stable expression in specific neuronal populations in vivo.

Endogenous protoxin modulators of nicotinic acetylcholine receptors

The cholinergic modulators lynx1 and lynx2 are a unique class of cell-surface regulatory molecules. These molecules are members of the Ly6 superfamily that also includes the three-finger fold snake venom toxins, α- and κ-bungarotoxin. Both lynx1 and lynx2 form stable associations with nicotinic acetylcholine receptors (nAChRs) and alter their function in vivo. Lynx1-like molecules are well conserved across species, both in structure and function, suggesting the importance of cell-surface modulators of nicotinic receptors in nature.

Cell-surface tethered toxin and peptide modulators

We have developed a novel strategy, termed “tethered toxins and peptides” to characterize neuronal circuits using the evolutionary derived selectivity of venom peptide toxins and endogenous peptide ligands, such as lynx1 prototoxins. Peptide toxins from predatory animals that have been routinely employed for neuroscience research do not normally exist as cell-surface anchored molecules. Using the scaffold of the lynx1-like gene family, i.e. secretory signal and consensus sequences for GPI processing and recognition, it is possible to produce a series of tethered toxins (t-toxins) that are highly effective modifiers of neuronal activity.



Applications of the tethered toxin and neuropeptide strategy. Endogenous peptide ligands, natural toxins, and synthetic peptides that are modified versions of ligands or toxins, can be integrated into recombinant membrane-attached fusion constructs and applied in vitro *in transfected or transduced cells in cell-culture, or* in vivo *in transgenic or virus-transduced animals. The t-peptide retains the specificity of the toxin/peptide ligand in a region or cell specific expression allowing for controlled manipulation of distinct subtypes of ion channels and receptors in a given neuronal circuit without affecting other channels/receptors in the cell.*

Approximately 40 different chimeric t-toxins derived from the venom of several predatory animals have been cloned and their activity characterized on voltage and ligand-gated ion channels. The t-peptide strategy has also been successfully extended to other bioactive peptides, such as ligand peptides for constitutive activation of GPCRs, illustrating the general applicability of this approach for cell-surface modulation of receptors (Figure).

Tethered toxins and peptides are being used for very diverse applications pertaining to experimental animal physiology. Because of their mode of action at the cell-surface, membrane-anchored peptide molecules act only on ion channels and receptors present in the membrane of the cell that is expressing the t-toxin or t-peptide, and not on identical receptors present on neighboring cells that do not express the tethered construct. We are applying the t-toxin strategy combined with transgenesis and viral-mediated genetic approaches for investigations regarding the physiology of neuronal circuits in the mouse nervous system.

Selected Publications

Auer S, Stürzebecher A.S., Jüttner R., Santos-Torres J., Hanack C., Frahm S., Liehl B., Ibañez-Tallon I. Silencing neurotransmission with membrane-tethered toxins. Nature Methods in press

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RNA Editing and Hyperexcitability Disorders

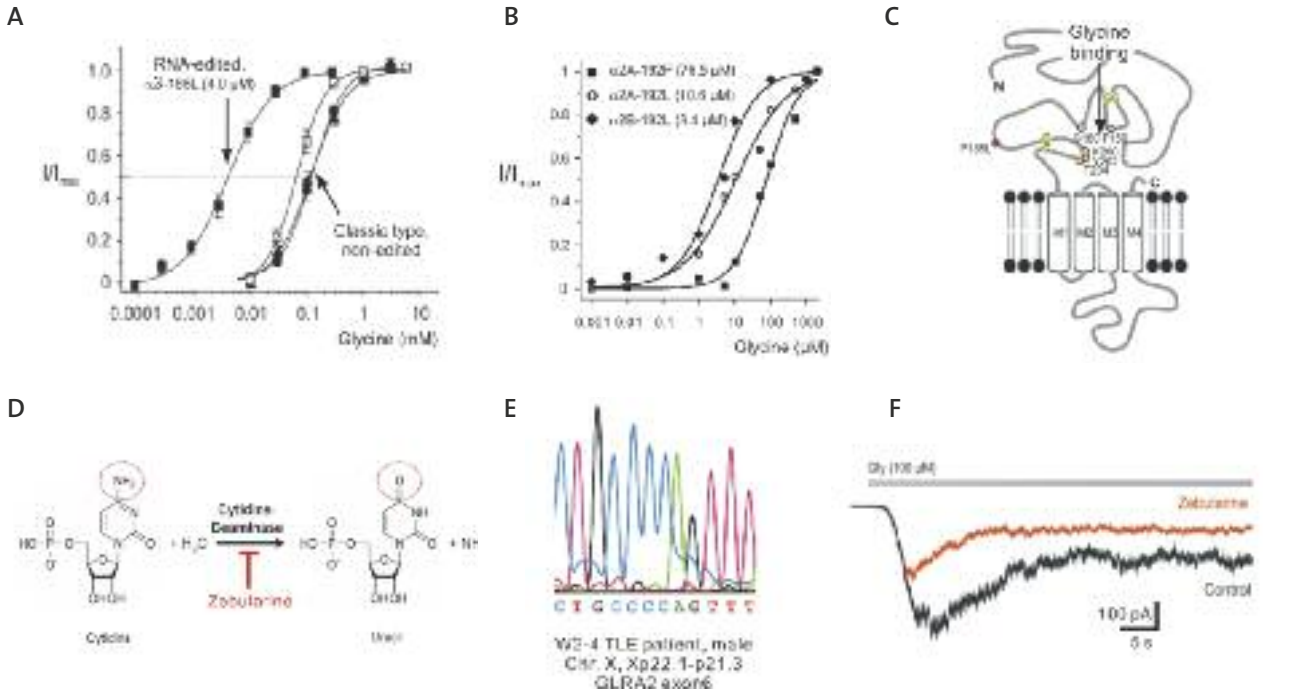
In a healthy organism, a balance is maintained between the excitation and inhibition of electrical impulses generated by neurons in the brain. Deregulation of this balance results in nervous system disorders. A core aspect of our work concerns the study of the brain at the molecular level, by investigating post-transcriptional enzymatic processes known in research as "RNA editing" and "splicing". We search for disease-associated alterations in post-transcriptional processes in the nervous system. Within this context, we are more closely scrutinizing glycine and GABA(A) receptors and gephyrin – key components of the molecular machine responsible for inhibition of electrical impulses in the brain.

Synaptic and tonic inhibition – Glycine receptor dynamics from the point of view of gephyrin

Neurotransmitter receptors are highly mobile entities within the neuronal plasma membrane. Enrichment of postsynaptic domains with neurotransmitter receptors therefore reflects a dynamic equilibrium between less mobile, synaptic and highly mobile, non-synaptic receptors. The diffusion rate is slowed down by reversible glycine receptor binding to the postsynaptic scaffolding protein gephyrin. These receptors contribute to synaptic inhibition of action potential generation whereas highly mobile receptors, which escaped postsynaptic anchoring, are involved in tonic inhibition of neuron firing. In the past, we could identify several novel splice variants of gephyrin, which were uncovered to adopt specific functions in the hippocampus. There, certain gephyrin splice variants were identified negative regulators of postsynaptic receptor stabilization at inhibitory synapses, providing us with novel experimental strategies for treatment of hyper-excitability disorders, such as temporal lobe epilepsy.

Deciphering the molecular basis of Molybdenum cofactor biosynthesis

Gephyrin has enzymatic activity. It is a multidomain protein that emerged from fusion of two bacterial proteins, MogA and MoeA. These *Escherichia Coli* proteins contribute to the biosynthesis of molybdenum cofactor (Moco), which is an essential component of cellular redox reactions. Mammalian gephyrins are still able to synthesize Moco because the enzymatic activity of the *E. Coli* homologous domains is preserved. In mammals, the most important Molybdenum enzyme is sulfite oxidase, which catalyzes the last step in the degradation of sulfur-containing amino acids and sulfatides. Human Moco deficiency is a hereditary metabolic disorder characterized by severe neurodegeneration resulting in early childhood death. We have identified a number gephyrin splice variants deficient in Moco synthesis. Therefore, another aspect of our work concerns the molecular and functional dissection of alternatively spliced gephyrins, using truncated and mutant expression constructs in a variety of cell types.



RNA editing emerges as a compensatory albeit pathophysiological mechanism in hyperexcitability disorders

Tonic inhibition of neuron firing plays a pivotal role in brain information transfer because it provides a global control of neuronal excitability. A large body of evidence has implicated impaired hippocampal GABAergic inhibition in enhanced susceptibility of neurons to become hyperexcitable and to generate epileptiform discharges. Glycine receptors have recently been involved in hippocampal tonic inhibition, and we could isolate mRNAs encoding gain-of-function glycine receptors with substantially increased apparent affinities for glycine (Figure A-C) and taurine, rendering them perfectly adjusted to mediating tonic inhibition. We could establish that high affinity glycine receptors arise from post-transcriptional C-to-U RNA editing (Figure D), as demonstrated by the absence of encoding genomic sequences (Figure E). Furthermore, the C-to-U RNA editing inhibitor zebularine was found to be effective on tonic glycinergic currents elicited in hippocampal neurons (Figure F). Having these tools at hand, functional analysis is carried out at a cellular level of investigation, using high affinity receptor expression in primary hippocampal neurons, and at a systemic level, using high affinity receptor screening of resected hippocampi from mesial temporal lobe epilepsy (TLE) patients. So far, our data point to a compensatory and homeostatic, but pathophysiological, role of high affinity glycine receptor activation in the course of TLE. Therefore, we are interested in developing this novel functional glycine receptor property into novel pharmacological approaches to the treatment of hyperexcitability disorders.

Selected Publications

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Björn Christian Schroeder

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Signaling and Transport Processes

The work of our group is aimed at understanding signal processes and epithelial transport on the molecular level. Currently we focus on the functional characterization of the recently identified TMEM16 family of ion channels with 10 members. Some of them are calcium activated chloride channels (CaCCs), known to be involved in various types of sensory transduction like vision, olfaction and pain perception, muscle contraction and blood pressure regulation as well as secretion of hormones and airway fluids. Mutations in one TMEM16 gene can cause Gnathodiaphyseal Dysplasia, a rare bone disease, while other family members are heavily unregulated in some cancer. It has also been suggested to use openers of TMEM16 channels to bypass the defect cystic fibrosis chloride channel in lung.

Introduction

CaCCs have first been described in salamander photoreceptors in 1982 and in many other cells in the following years. They activate in response to elevated levels of calcium ions in cells. The effect of the chloride conductance depends on the intracellular chloride concentration. Its opening can cause depolarization, faster repolarization, the prevention of action potentials or chloride secretion. Their function has been studied extensively in some cell types. E.g. in olfactory receptor neurons they act as an amplifier to ensure neuronal depolarization. The role in some smooth muscle cells is to produce depolarization that opens voltage dependent calcium channels to produce contraction, and in many epithelia, e.g. salivary and lacrimal glands, chloride secretion through CaCCs drives fluid secretion.

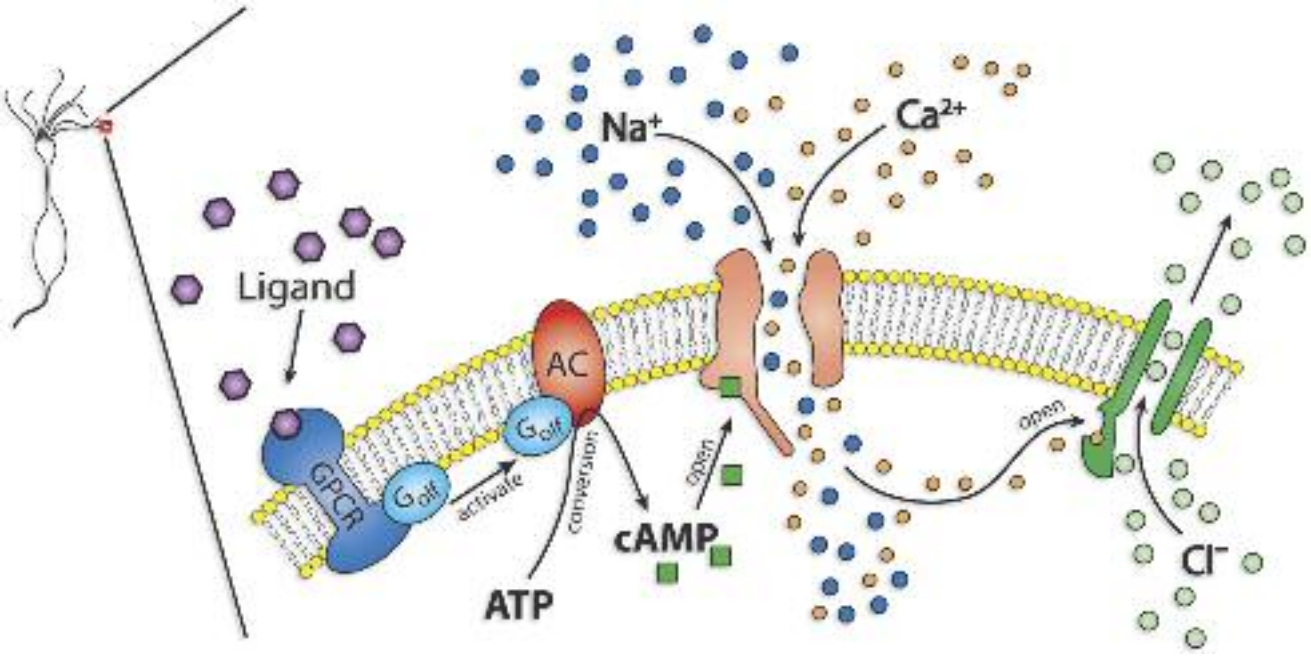
However the molecular identity of these channels remained elusive over the last decades. In 2008, as a postdoctoral researcher at the University of California – San Francisco, my research lead to the discovery that members of the TMEM16 family of membrane proteins encode CaCCs – making TMEM16s the latest addition to the small group of ion channel families.

So far two members of the TMEM16 family, TMEM16A and TMEM16B, have been shown to encode CaCCs. Their function and regulation can now be studied on the molecular level. In addition we are interested in the role of the other members of the TMEM16 family.

Structure / function and cell physiology of TMEM16 proteins

To perform structure and function studies on the TMEM16 family using site-directed mutagenesis a transient expression system is required. A very convenient one is the oocyte system in combination with two electrode voltage clamp. It allows screening of many genetically modified ion channel constructs in short time. We now establish oocytes of the salamander *Ambystoma* mexicanum as alternative to the traditionally used *Xenopus* oocytes. The later ones can not be used with CaCCs as they, in contrast to salamander oocytes, express large amounts of endogenous CaCCs. We have generated several TMEM16 mutants, which will be analyzed with this system as well as in transfected HEK-293 cells using patch clamp techniques.

Some of the constructs we generated are fusion proteins with the fluorescent reporter's eGFP and cherry. We use them to search for changes in the sub cellular localization of TMEM16 proteins after various stimuli or after coexpression with potential interaction partners.



CaCC in olfactory receptor neurones *Binding of odorants to specific G-protein coupled receptors activates an adenylate cyclase (AC) through the G-protein β-subunit Golf. The produced cAMP binds to and opens cyclic nucleotide gated channels and calcium enters the cell. The initial depolarization caused by the opening of this unselective cation channels is than amplified by the opening of CaCCs. It has been estimated that the chloride current can be up to 30 times larger than the cation current.*

We generated antibodies against TMEM16A and other members of the family and could show that TMEM16A and F show highly polarized expression in epithelial cells and some neurons. This is consistent with calcium activated chloride currents in native cells which display in an asymmetric fashion.

TMEM16 in vivo studies

To understand the in vivo function of TMEM16 proteins and their relationship to CaCCs we, in collaboration with the group of Christian Hübner, University of Jena, have started to generate TMEM16 knock out mice. To do this we have used floxed constructs. This will give us the option of a tissue specific knock out by crossing the floxed lines with appropriate Cre lines. Currently, it is only possible to speculate about the experiments necessary for the analysis as they will depend mainly on the observed phenotypes. For the analysis we plan to test physiological functions known or hypothesized to depend on CaCCs including olfaction, taste and blood pressure regulation. In addition we are interested in the role of TMEM16A in transepithelial water- and ion transport of the lung. Its expression in airway epithelia makes it a good candidate to be the channel that could bypass the defective CFTR chloride channel in patients suffering from cystic fibrosis.

We hope that our research will not only help to understand the physiological role of CaCCs, but also lead to new treatments of diseases like hypertension, asthma or cystic fibrosis.

Selected Publication

Schroeder, BC, Cheng, T, Jan, YN, Jan, LY. (2008). Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell. 134, 1019-1029.

Jan Siemens
(Sofia Kovalevskaya Prize)

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Sensory Neurobiology

The ability of any living organism to probe and sense stimuli emanating from the surrounding environment is of fundamental importance for its well-being and survival. While a lot of progress has been made in elucidating sensory systems such as vision and olfaction, our understanding of the somatosensory system, giving rise to the detection of (painful) mechanical stimuli as well as changes in temperature, is lacking behind.

Sensory mechanisms not only monitor the outside world but also detect changes of interior parameters such as blood sugar levels, osmolarity, body temperature and many others. Processing and integration of sensory information from the outside world and the interior environment allows our body to maintain homeostasis.

Our research focuses on understanding sensory mechanisms at the molecular level.

Previous Work

As a postdoctoral fellow in the lab of David Julius at the University of California San Francisco, I focused on thermo-sensory ion channels of the pain pathway. My projects have centered on describing new pharmacological tools to study and manipulate these ion channels and on genetic methods to determine their physiological roles *in vivo*.

Bites and stings from venomous creatures can produce pain and inflammation but little is known of how venom ingredients activate the pain pathway. In the somatosensory system, sensors for painful (and thermal) stimuli include the TRP channel family members TRPV1, TRPM8, and TRPA1. I found that three **i**nhibitor **c**ystein **k**not (ICK) peptides from tarantula venom target TRPV1, the capsaicin receptor. In contrast to the predominant role of other ICK toxins as channel inhibitors, these newly discovered toxins -termed vanillotoxins- function as TRPV1 agonists, which explains how venoms can cause pain. Moreover, these toxins constitute new pharmacological tools to study TRP channel gating and generate drugs that block the pain pathway.

TRPM8 is activated by menthol, a product of the mint plant used in chewing gum and toothpaste to evoke a cooling sensation. Low temperatures activate TRPM8 *in vitro*, further suggesting that TRPM8 is a cold receptor. To test this hypothesis *in vivo*, we generated TRPM8 deficient mice and examined their response to cold stimuli. We found that these mice were not able to discriminate between cold and warm temperatures in behavior assays and displayed dramatically reduced responses to cold stimuli at the cellular level. These findings validate the hypothesis that TRP channels participate in the sensation of thermal stimuli in the peripheral nervous system.

TRPA1 is a detector of environmental irritants such as acrolein, an airway irritant present in tear gas, vehicle exhaust and smoke. We found that 4-Hydroxynonenal (HNE), a molecule produced in response to tissue injury, is a potent activator of TRPA1, demonstrating that this ion channel is also an important sensor for endogenous inflammatory factors.

In summary, my postdoctoral studies have advanced our understanding of TRP channel function in inflammation and peripheral temperature sensation.

Current Research

I remain fascinated by mechanistic aspects of sensory neuroscience and my research team will continue to focus on understanding sensory mechanisms at the molecular level. We are investigating 3 areas in this field:

Identification of sensory TRP channel modulators.

By analogy to TRP channels in the *Drosophila* eye, we hypothesize that the mammalian orthologs are components of supramolecular membrane-bound protein complexes that enable the channels to function specifically and effectively in a context dependent manner. We are using genetic, biochemical and functional screening paradigms to identify components of this putative modulatory complex.

Identification of molecules involved in developmental and functional aspects of mechanosensation.

The ability of cells to detect and transduce mechanical stimuli is a fundamental biological process that underlies touch, pain and control of muscle tension. In vertebrates, a subpopulation of somatosensory neurons is specialized to detect these different mechanical stimuli but how they accomplish this task is at present not understood. Similarly, the developmental program that allows these neurons to differentiate into a diversity of cell types with very specific mechanosensitive traits is largely unknown.

We are employing a multidisciplinary approach geared towards the identification and characterization of molecules that are necessary for the development and function of mechanosensory neurons. Using the mouse as a model system, we have conducted a genetic screen that will allow us to isolate molecules such as transcription factors and signaling molecules involved in the differentiation of mechanosensory neurons. Subsequently, we will test their potential to drive differentiation of neuronal precursors into specific mechanosensory neurons using stem cell biology.

Molecular mechanisms underlying temperature-detection and core body temperature regulation.

Temperature detection and regulation is of vital importance to any homeothermic organism. In order to maintain temperature homeostasis it is necessary for the autonomic nervous system to monitor small fluctuations in core body temperature and initiate counter measures to prevent temperature fluctuations beyond a tightly controlled set point. Key brain centers concerned with temperature control are the preoptic area and anterior hypothalamus (PO/AH). These hypothalamic regions harbor neurons that not only detect changes in core body temperature, but are also believed to receive and integrate input from ascending somatosensory pathways carrying information from peripheral temperature sensors.

The molecular machinery underlying central temperature detection by hypothalamic neurons is currently unknown. We will characterize the thermal response properties generated by hypothalamic PO/AH neurons, with the goal of elucidating mechanisms underlying their temperature sensitivity.

The picture depicts Embryonic Stem (ES-) cells that have been differentiated into neuronal precursors in vitro. *The cells have been stained with antibodies for the neurofilament nestin (red), the cell proliferation marker Ki-67 (green). The cell nuclei are labeled by Dapi (blue). Our goal is to differentiate these precursors into sensory neuron lineages, thereby generating largely homogenous cell populations that can be used for biochemical experimentation.*

Selected Publications

Siemens, J, Zhou, S, Piskorowski, R, Nikai, T, Lumpkin, EA, Basbaum, AI, King, D, Julius, D. (2006). Spider Toxins activate the Capsaicin Receptor to produce Inflammatory Pain. Nature. 444, 208-212.

Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, Jordt SE, Julius D. (2007). The Menthol Receptor TRPM8 is the Principal Detector of Environmental Cold. Nature. 448, 204-208.

Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imamachi N, Andrè E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D, Geppetti P. (2007). 4-Hydroxynonenal, an Endogenous Aldehyde, causes Pain and Neurogenic Inflammation through Activation of the Irritant Receptor, TRPA1. Proc Natl Acad Sci. 104, 13519-24.

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Neural Circuits and Behavior

Recent technical advances are allowing researchers to make high resolution recordings and manipulations of brain activity even while animals are awake and behaving. These data are providing remarkable insights into normal brain function. Of particular interest is the activity of neurons in neocortex, as they are involved in conscious movement and sensory perception. Our lab combines electrophysiological and optical neural recordings with genetically targeted manipulations of neural activity in mice during trained behavior to investigate the link between neural activity, sensory perception and motor behavior.

Brain states during behavior

The very first recordings from the awake human brain by Hans Berger (1929) revealed distinct patterns of cortical activity during different behavioral states. Different "brain states" were thought to reflect changes in the synchrony of cortical activity and be fundamental to sensory perception, sensorimotor coordination and learning. However, it was unknown how cortical synchrony is reflected in the intracellular membrane potential (V_m) dynamics of behaving animals. With Carl Petersen (EPFL, Switzerland) we showed, using dual whole-cell recordings from layer 2/3 primary somatosensory mouse whisker barrel cortex, that the V_m of nearby neurons is highly correlated while the mouse is sitting still. However, when the mouse moves its whiskers, to sense the environment, an internally generated state change reduces the V_m correlation (Figure 1).

We went on to show that the rate of action potential firing was surprisingly low in layer 2/3 pyramidal neurons. Single action potentials were driven by a large, brief and specific excitatory input that was not present in the V_m of neighbouring cells. Action potential initiation occurs with a higher signal-to-noise ratio during whisker movement as compared to quiet periods. The change in brain state therefore may increase the overall information coding capacity of the brain.

We have gone on to investigate the neural mechanisms underlying the change in brain state. So far we have shown that the state change is not the result of sensory feedback as it persists even after cutting the primary sensory neurons that innervate the whisker pad (Figure 2). We are presently investigating the role of the thalamus in regulating cortical state changes.

Sensorimotor integration in the mouse forepaw cortical region

Our lab will focus on the primary sensory and motor cortical areas associated with mouse forepaw. Neuronal activity in cortical regions controlling limb movement is particularly relevant in the emerging field of neuroprosthetics, where cortical activity from paralyzed patients is used to drive robotic limbs. We will train mice to make targeted reaching movements while recording and manipulating cortical neural activity.

Role of acetylcholine in cortical processing and behavior

The neocortex is densely innervated by axon fibers containing acetylcholine (ACh). Neurons containing ACh have received much attention, perhaps mainly because of their degradation in Alzheimer's disease but also due to their putative role in cognitive processing such as

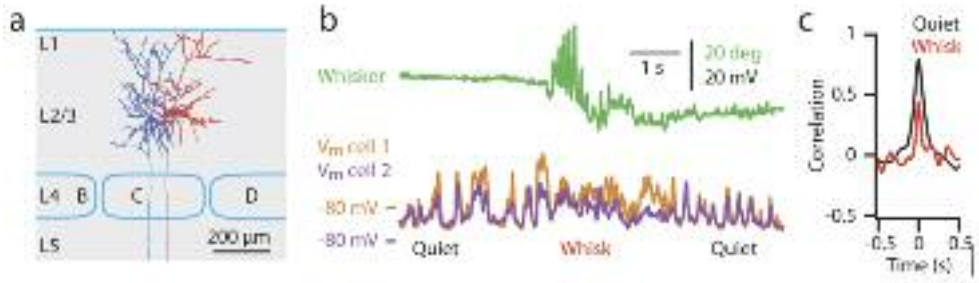

Figure 1. *(**a**) Anatomical reconstruction of two layer 2/3 pyramidal neurons in mouse barrel cortex. (**b**) Simultaneous dual whole-cell recording of cells shown in A while the mouse is sitting still (quiet) and during whisking movements (whisk). (**c**) Cross correlation of V_m in these two neurons shows high correlation during quiet periods and a reduced correlation during whisker movements. (from Poulet and Petersen, 2008).*

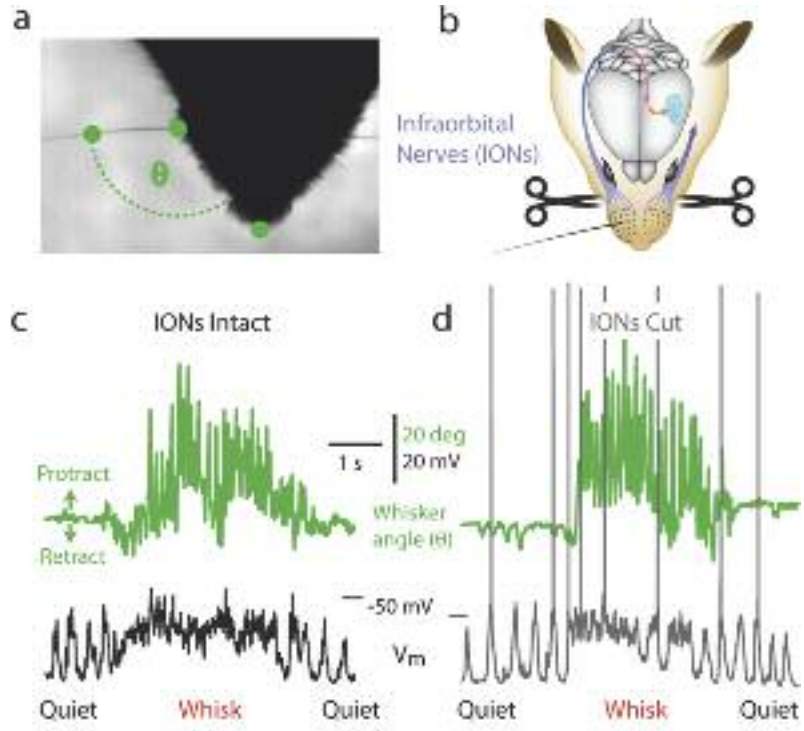

Figure 2. *(**a**) Single image from high speed (500Hz) whisker filming showing measurement of whisker angle (b) Schematic of whisker sensory pathway, scissors indicate position of bilateral ION cut. (**c**) Membrane potential (V_m) dynamics undergo a change in state during whisking (red) as compared to quiet periods (black), irrespective of whether the IONs were intact (black) or (**d**) cut (gray).*

memory, attention, plasticity and modulation of sensory responsiveness. Despite the widespread and important roles of ACh, the underlying *in vivo* circuit and synaptic mechanisms of its action in the central nervous system have remained elusive. In this project, we will selectively manipulate the activity of ACh neurons using novel genetically-targeted techniques to reveal the precise role of ACh in cortical processing and ultimately behavior.

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Poulet JFA and Hedwig B (2002) A corollary discharge mechanism maintains auditory sensitivity during sound production. Nature 418, 872-876.

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Dr. Raluca Niesner

Molecular Neurology

The group works on immune regulation, neural cell damage and neuronal protection mechanisms in the context of inflammatory pathologies of the nervous system such as multiple sclerosis. Methodology ranges from murine and human lymphocyte and brain slice culture to models of neuroinflammation, in vivo multi-photon-microscopy and magnetic resonance imaging. The overall goal is to translate resulting knowledge into proof-of-concept clinical trials. The work is mainly performed within two collaborative research centers: SFB-TRR 43 and SFB 650.

The role of the immune system in inflammatory neurodegeneration and repair

Neuronal damage has only recently been shown to influence the pathology in inflammatory diseases of the central nervous system such as meningitis and multiple sclerosis (MS). We have meanwhile experimental and clinical data for a marked loss of lower motor neurons in MS patients. We regularly find dying spinal motor neurons surrounded by CD3+ (CD4+ as well as CD8+) T cells expressing tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) in post-mortem tissue from MS patients. Based on these findings and on experimental data in experimental autoimmune encephalomyelitis (EAE), our study indicates that damage to lower motor neurons and TRAIL-mediated inflammatory neurodegeneration in the spinal cord contribute to MS pathology (Vogt et al. 2009).

Performing two-photon laser scanning microscopy in the brainstem of living mice suffering from EAE, we furthermore visualize T cells migrating into the central nervous system (CNS) and directly attacking neurons and their processes. Both in mouse and man, we have very recently in collaboration with M. Bader shown that a system known to play a role in cardio-vascular medicine, namely the kallikrein-kinin-system, is of relevance for the transmigration of proinflammatory T cell subsets into the CNS (Schulze-Topphoff et al, in press). Bradykinin receptor 1 inhibits infiltration, especially of T helper 17 cells (TH17) which are proinflammatory and encephalitogenic. Prior to their effector function, the T cells exert distinct behavior at the blood brain barrier with CD4+ T cell compartmentalization in the parenchyma along CNS vessels. This process is dependent on the chemokine CXCR4, while key adhesion molecules seem to have no major function in this process (Siffrin et al. 2009). This is in contrast to their essential role in the transmigration step, in which integrin-mediated adhesion is necessary to overcome endothelial barriers. Within the perivascular compartment, the lymphocytes can interact with each other and/or other immune cells as a prerequisite for the sequential steps involved in the process of inflammation and immune regulation. Collectively, these findings support the idea that compartmentalization of activated CD4+ T lymphocytes in the target organ serves a similar purpose as in lymph nodes, i.e. increasing the chance of T cell receptor and co-receptor engagement with the whole spectrum of possible outcomes between tolerance and immunity.

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With regard to potential repair properties of the inflamed CNS, we have studied the cell-fate decision of neural progenitor cells (NPC) being affected by the redox state with oxidizing conditions favouring differentiation in astrocytes, whereas reducing conditions favour neuron formation. To analyse the molecular mechanism underlying these effects, we investigated a gene called *Hairy/enhancer of split 1* (*Hes1*), a transcriptional repressor of *Mash1*, which, in turn, is responsible for the activation of a neuron-specific transcription program. We found that under oxidizing conditions, the histone deacetylase Sirt1 and Hes1 form a complex that binds to and deacetylates histones at the *Mash1* promoter. These events cause downregulation of Mash1 expression and block neuronal differentation. In a reducing environment, the Hes1–Sirt1 complex is not observed. Instead, Hes1 recruits transcription activators such as CREB binding protein to the Mash1 promoter, and this drives NPC towards a neuronal fate (Prozorovski et al. 2008). The influence of the redox state on NPC cell-fate decisions was eliminated by removal of Sirt1 activity either by RNAi or through the use of Sirt1 inhibitors. Thus, through its action at the *Mash1* promoter, Sirt1 seems to act as a cell-fate decision switch in NPCs *in vitro*, with increased Sirt1 activity causing increased differentiation of NPCs into astrocytes at the expense of neurons. Using healthy mice and EAE, *in vivo* investigations followed. Injecting young mice with an oxidizing agent increased the number of newly differentiated Sirt1-positive astrocytes in the brain. Performing *in utero* electroporation of GFP-marked RNAi constructs against Sirt1 to deplete Sirt1 in collaboration with Robert Nitsch, an increased proportion of Mash1-positive cells was found in postnatal animals treated with an oxidizing agent. EAE induces an oxidizing environment and reactive astrogliosis. Whereas Sirt1 expression was low in unaffected brain regions, inflamed areas with an abundance of invading leukocytes contained an increased number of cells positive for both GFAP and Sirt1.

Selected Publications

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Cellular Neurosciences

Our goal is to understand the role of glial cells in physiology and pathology. We focus on questions as to how neuronal activity is sensed by astrocytes, how astrocytes communicate among each other, and how they feedback on neurons. A second focus addresses the role of connexin proteins in oligodendrocytes function, the cells which are the myelin forming cells in the brain. Thirdly, we study the expression of transmitter receptors in microglial cells and how activation of these receptors influences microglial function. This is of particular interest within the context of pathology and we are currently studying this question in stroke and gliomas. A fourth line of research addresses the question as to how glioma cells interact with the intrinsic brain cells, specifically microglia and stem cells. We are aiming to understand this interaction on a molecular level, in particular with the hope of identifying tools which impair glioma invasion.

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 the neuronal membrane. They form intimate contact with synapses and neuronal activity results in astrocyte responses. Microglial cells are immuno-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 role of astrocytes in information processing (2) the impact of connexion expression for oligodendrocytes function (3) the response of microglial cells to brain injury and (4) the interaction of gliomas with microglia and stem cells.

Mechanisms of neuron-astrocyte interactions

This project aims to understand signaling mechanisms between astrocytes and neurons. We recently have focused on two preparations, the barrel cortex and the medial nucleus of the trapezoid body. The Calyx of Held is a giant glutamatergic terminal on the principal neurons in this nucleus. It has been used as a model synapse to study mechanisms of transmitter release and synaptic plasticity since both, pre- and postsynaptic elements can be simultaneously recorded using physiological techniques. We have studied the morphological arrangements and the properties of the astrocytes which are in close contact with the Calyx. We use brain slices containing the medial nucleus of the trapezoid body and have established to simultaneously record

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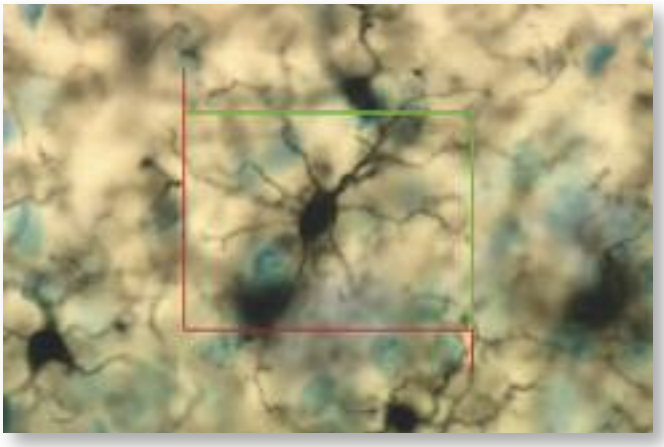

Figure 1. *Stereological counting of microglial cells in the dentate gyrus of B6/129svj mouse. Microglia are stained with anti-Iba1 antibodies and visualized with DAB (Olympus, 100x). Nuclei are counter-stained with toluidine blue.*

from neurons and astrocytes. We obtained evidence that two types of astrocytes perceive the Calyx activity. One type of astrocyte is characterized by a complex membrane current pattern and these cells receive synaptic input mediated by glutamate. The other type of astrocyte characterized by a passive membrane current pattern exhibits currents which are due to glutamate uptake. Ultrastructural inspection revealed that both types of astrocytes are in direct contact with both, the pre- and postsynaptic membrane. Moreover, we could identify glial postsynaptic structures on the cell with complex current pattern. One goal of this study is to determine how astrocytes integrate synaptic input from defined synapses (funded by a Schwerpunktprogramm of the DFG).

The sensory input of the whiskers in rodents is represented in the somatosensory cortex. Each whisker projects into a defined cortical area, the barrel field. These areas are morphologically delineated and can be recognized in acute brain slices without additional staining. The barrel cortex is a well established model for plasticity since removal of whiskers results in changes of the barrel fields. After stimulation in the cortical layer 4, the input to the barrel field, we can record responses in astrocytes and in neurons by using Ca^{2+} imaging and patch-clamp recording. While the neuronal activity spreads beyond barrel borders, the astrocyte activity is restricted to the barrel field. We are now particularly interested in the question how astrocyte activity feeds back on neuronal activity. In an ongoing study we try to perturb astrocyte function while simultaneously recording neuronal activity.

How does connexin expression affect oligodendrocyte function?

In a collaboration with Prof. Klaus Willecke's group in Bonn we are studying the expression of connexins, the molecular substrate for gap junctions in oligodendrocytes. Gap junctions are communication channels between cells which allow the exchange of molecules between cells, but also serve for intercellular signaling. They can also connect different compartments within a given cell. By using different combinations of mouse lines with connexion deletions, we determine which of the connexins are essential to form gap junctions among oligodendrocytes and among astrocytes and oligodendrocytes. This also has an important clinical impact. Mutations in connexins expressed by oligodendrocytes can lead to defects in myelin formation, an important function of oligodendrocytes. We are currently studying a mouse mutant which mimics a patient mutation which leads to leukodystrophy. We

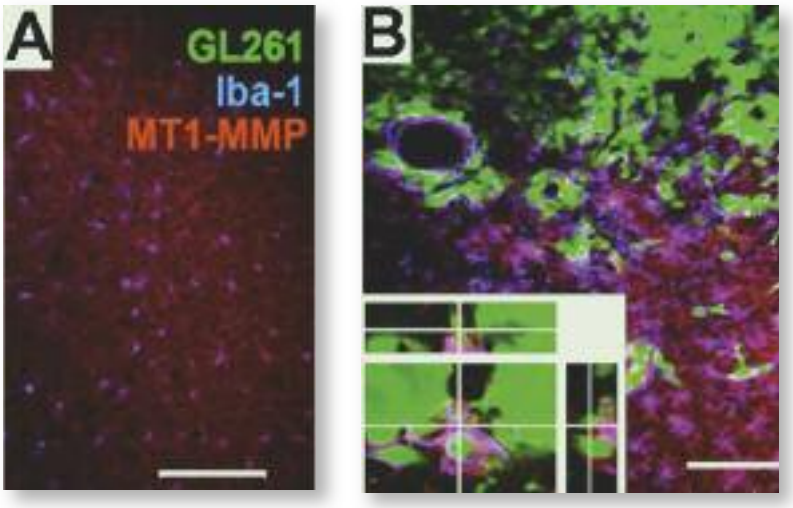

Figure 2. ***Iba1 positive microglia cells over-express MT1-MMP when associated with experimental gliomas.*** *Mouse brains injected with GL261 glioma cells expressing EGFP (green) were studied for the microglia marker Iba1 (blue) and for the metalloprotease MT1-MMP (magenta). (**A**) In the control (glioma non-injected) hemisphere, the level of MT1-MMP expression is low. (**B**) The density of microglial cells is much higher in the vicinity of gliomas than in the normal brain; moreover, MT1-MMP is over-expressed in microglia associated with gliomas; labelling for MT1-MMP is especially intense in microglia making close contact with glioma cells, whereas GL261 glioma cells express only very low levels of MT1-MMP; a magnified 3D reconstructed micrograph of the tumor area is shown in the insert.*

are, therefore, interested in the question how coupling determines myelin formation and maintenance (Funded by Deutsche Forschungsgemeinschaft).

What are the physiological features of microglial cells in brain tissue?

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. They also express a variety of receptors for chemokines and cytokines as expected from a macrophage-like cell. We have addressed the question whether microglia would also express receptors to sense neuronal activity. We have recently developed an in situ model which allows us to study the physiological responses of resting and activated microglia. This enables us to characterize the functional receptors and the physiological phenotype of microglia in situ. Using this approach, we could identify microglial receptors for GABA, the major inhibitory transmitter of the CNS. Activation of the $GABA_B$ receptors suppressed indicators of microglial activation such as the release of IL-6. A similar reduction in proinflammatory mediators was found with activation of purinergic receptors and of adrenergic receptors. GABA also triggers an indirect effect on microglia since GABA receptor activation in other glial cells and neurons trigger an elevation in extracellular potassium which is sensed by microglial cells. This leads to an enhanced release of the cytokine MIP-1α.

Microglia expresses a variety of purinergic receptors and the expression pattern undergoes changes during development and in pathology. We have found an interesting interplay between purinergic and adenosine receptors to control microglial migration. In the extracellular space, ATP is rapidly degraded to ADP, AMP and adenosine. In the brain, two prominent ectonucleotidases, cd39 (NTPDase1) degrading ATP to AMP and cd73 (5`-nucleodidase) degrading AMP into adenosine, are exclusively expressed by microglial cells and even have served as microglial-specific markers. We found that ATP fails to migration in microglia deficient for cd39. However, the effects of ATP on migration in cd39 deficient microglia can be restored by co-stimulation with adenosine or by addition of a soluble ectonucleotidase. We also tested the impact of cd39 deletion in a model of ischemia, in an entorhinal cortex lesion and in the facial nucleus after facial nerve lesion. The accumulation of microglia at the pathological sites was markedly decreased in cd39 deficient animals. We conclude that the co-stimulation of purinergic and adenosine receptors is a requirement for microglial migration and that the expression of cd39 controls the ATP/adenosine balance (funded by DFG).

Do microglial cells influence glioma cells?

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. 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

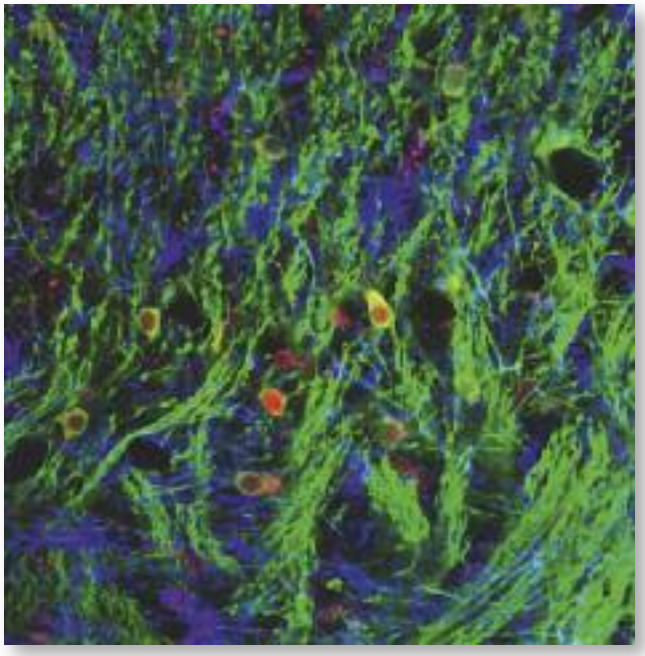

Figure 3. *Oligodendrocytic coupling in the corpus callosum of WT mice. Confocal image of the biocytin/streptavidin-Cy3 (red) labelled network immunostained for the oligodendroglial markers NG2 (blue) and CNPase (green). A single oligodendrocyte was injected with the gap junction permeable tracer biocytin by whole-cell patch clamp. Biocytin staining revealed a network consisting of 39 cells. The majority of coupled cells was identified as CNPase expressing oligodendrocytes, only 5% as NG2 positive while 13% was negative for both markers. Within the CNPase positive population 42% of the coupled cells were weakly expressing NG2.*

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 toll-like receptors in the surrounding microglial cells and triggers the expression of the MT1-MMP. Thus, glioma cells exploit microglial cells to promote their invasion. Glioma cells obviously need microglia since they can not produce MT1-MMP themselves: A forced expression MT1-MMP in glioma cells leads to their death. 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.

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Do stem cells influence glioma cells?

We have previously observed that gliomas attract neural precursor cells from the subventricular zone. These cells migrate over large distances and enwrap the tumor yet they do not originate from the tumor proper as was previously suspected. This intrinsic anti-tumorigenic response is strongly related to age in an animal model and occurs only during youth when neural precursor cells are more active. Consequently, in older animals this interaction does not occur. The precursor cells inhibit tumor growth and addition of exogenous precursors prolongs the survival rate in older animals. The proliferative response of NPCs to glioblastomas depended on the expression of D-type cyclins. In young mice, NPCs express the cyclins D1 and D2, but the expression of cyclin D1 is lost during aging, and in adult NPCs only cyclin D2 remains. In young and adult cyclin D2-deficient mice we observed a reduced supply of NPCs to glioblastomas and the generation of larger tumors compared with wild-type mice. We conclude that cyclin D1 and D2 are nonredundant for the antitumor response of subventricular NPCs. Loss of a single D-type cyclin results in a smaller pool of proliferating NPCs, lower number of NPCs migrating to the tumor, and reduced antitumor activity. We are currently characterizing factors by which stem cells suppress tumor growth. Interestingly, there are two distinct mechanisms, one targeting the glioma cell mass inducing cell death. The second mechanism is targeted towards glioma stem cells, a small population of cells within the tumor mass identified by the antigen CD 133. These cells are highly aggressive and are therefore novel targets for therapeutic approaches.

This research is funded by a binational BMBF grant with Bozena Kaminska, Warsaw (DLR 01GZ0701).

Selected publications

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Pocock, JM and Kettenmann H. (2007) Neurotransmitter receptors on microglia, Trends Neurosci. 30, 527-535.

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Proteomics and Molecular Mechanisms of Neurodegenerative Disorders

Proteins are central to almost all cellular functions, such as metabolism, regulation of cell growth and communication between cells. They are synthesized from amino acid building blocks and must fold into unique three-dimensional structures in order to become functionally active. Misfolded proteins are a normal feature of this process. Under physiological conditions misfolded proteins are efficiently degraded. When cells are challenged with environmental stress or genetic mutations, however, protein misfolding increases dramatically and changes various cascades of cellular function, which leads to the manifestation of pathological phenotypes. More than 35 systemic and neurological diseases are caused by the formation of abnormally folded protein species, among them the late-onset neurodegenerative disorders Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). To date, only symptomatic treatments with limited effectiveness are available for these illnesses.

The main objective of our work is to elucidate the molecular mechanisms of protein misfolding diseases. Several lines of experimental evidence indicate that abnormal protein assembly *in vitro* and *in vivo* is a multistep process, involving the formation of on- and off-pathway aggregation intermediates such as spherical oligomers or worm-like protofibrils. However, the structure, size and morphology of these potentially highly toxic structures have not been fully clarified. Moreover, it remains unclear how misfolded protein species interact with their cellular environment and cause cellular dysfunction and toxicity. Evidence was presented recently that abnormal protein aggregates alter the function of complex cellular processes such as membrane signaling or protein degradation mediated by the ubiquitin proteasome system (UPS). However, the impact of these changes on cell function remains largely unknown. Also, it remains unclear why certain types of cells accumulate misfolded proteins while others do not.

Chemical compounds that are able to modulate protein misfolding pathways *in vitro* and *in vivo* are highly valuable tools to analyze the complex protein assembly process. They are valuable starting points for therapy development. Besides small molecules we are also highly interested in finding proteins or peptides that directly influence the amyloid formation cascade. They are identified using high throughput protein interaction technologies such as an automated yeast two-hybrid (Y2H) system. Recently, a network of dysregulated protein-protein interactions (PPIs) for HD was created using bioinformatic strategies. This study allowed the identification of the neuron-specific protein CRMP1, a protein that dramatically influences polyglutamine (polyQ)-mediated huntingtin aggregation in cell-free and *in vivo* disease model systems.

Finally, we aim to develop innovative technologies and databases for systematic proteomics research. An interactome database termed UniHI was established at the MDC that contains more than 250,000 human interactions and allows scientists to link individual proteins to ing cascades and disease processes.

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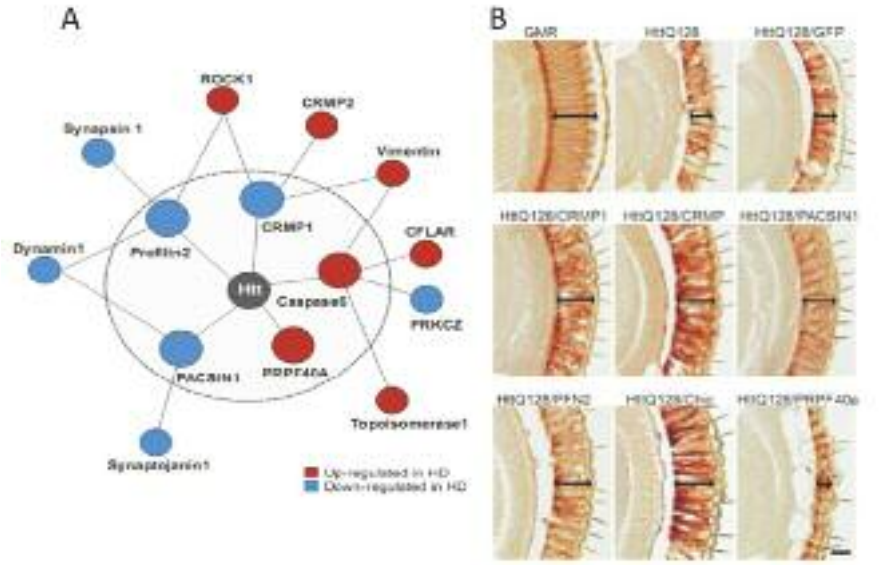

Systematic investigation of predicted dysregulated huntingtin interaction partners in a HD Drosophila model. (A) Dysregulated huntingtin PPI network predicted by bioinformatic analysis of PPI and gene expression data from human tissues and clinical case-control studies. (B) Expression of the proteins CRMP1, CRMP, PACSIN1, Profilin2 and Chic reduce mutant huntingtin-mediated photoreceptor degeneration in a HD model, whereas expression of the protein PRPF40a has the opposite effect.

Identification and characterization of small molecules that modulate protein misfolding pathways

In previous studies, we have identified the natural compound (-)-epigallocatechin gallate (EGCG) as a modulator of polyQ-mediated huntingtin aggregation (Ehrnhoefer et al., 2006). We continued these studies with the disease proteins causative of PD and AD. α-synuclein and amyloid-β amyloidogenesis was investigated using biochemical, biophysical and cell biological methods. We found that EGCG efficiently prevents fibril formation of both polypeptides in cell-free model systems, supporting the previous results with the huntingtin protein and hinting at a generic mechanism. Further studies subsequently revealed that EGCG is able to redirect the amyloid fibril formation pathway. It binds to α-synuclein and amyloid-β monomers and stimulates the assembly of off-pathway, highly stable oligomers, which are non-toxic for mammalian cells. These investigations suggest that EGCG functions as a chemical chaperone that recognizes natively unfolded polypeptides and influences their ability to spontaneously misfold and self-assemble into amyloidogenic protein aggregates (Ehrnhoefer and Bieschke, 2008).

Most recently, we studied whether EGCG disassembles preformed amyloid fibrils. Our data indicate that EGCG has the ability to convert preformed, mature α-synuclein and amyloid-β fibrils into smaller, amorphous protein aggregates that are non-toxic for mammalian cells. These findings suggest that EGCG is a potent remodelling agent of mature amyloid fibrils and support our hypothesis that the compound has chemical chaperone function.

Several lines of experimental evidence indicate that soluble, pre-fibrillar assemblies of the amyloid-β polypeptide rather than mature, end-stage amyloid fibrils cause neuronal dysfunction and memory impairment in Alzheimer's disease. This suggests that acceleration of fibrillogenesis might reduce the levels of toxic aggregation intermediates. To address this question, we searched for chemical compounds that promote spontaneous amyloid-β formation. Using a filter assay, the natural dye orcein and related substances were identified which directly bind to small transient amyloid oligomers and stimulate their assembly into mature amyloid fibrils. These structures have altered surface properties and are non-toxic for mammalian cells. Our results suggest that conversion of small

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aggregation intermediates into large amyloid structures might be a powerful therapeutic approach to treat protein misfolding diseases.

In order to investigate whether the small molecules that modulate amyloid formation pathways *in vitro* and *in vivo* are useful for therapy development, a drug discovery program for AD and HD was started in 2007 in the framework of the GO-Bio initiative of the German Federal Ministry for Education and Research. The aim of this program is to promote the establishment of innovative start-up companies out of the academic sector. In the first funding phase of the Go-Bio project, we have discovered a number of novel drug candidates for both AD and HD and developed them up to *in vivo* proof of concept in transgenic mouse models of the two diseases. At the moment, derivatisation and further *in vivo* testing are in progress in the framework of lead optimisation.

Identification of proteins that modulate polyQ-mediated huntingtin aggregation

HD is an inherited neurodegenerative disorder that is caused by an expansion of a polyQ tract in the protein huntingtin, which leads to a characteristic accumulation of insoluble Htt aggregates in affected neurons and eventually to cellular dysfunction and toxicity. However, the molecular pathways underlying brain-specific, polyQ-induced neurodegeneration in HD are still unknown. Recently, a large number of interaction partners were identified that associate with the N-terminal domain of huntingtin, which harbours the aggregation-prone polyQ tract. We hypothesized that perturbation of functional huntingtin protein complexes in neurons induces protein misfolding and neurotoxicity. To identify tissue-specific, dysregulated huntingtin protein interactions, a bioinformatic approach was developed. By filtering publically available protein-protein interaction (PPI) data with information from gene expression studies of brain and non-brain tissues as well as clinical case-control studies, a brain-specific huntingtin PPI network was created, linking 14 potentially dysregulated proteins directly or indirectly to the disease protein. Analysis of published data confirmed the predictive value of this network modelling strategy. Moreover, systematic investigations with *in vitro* and *Drosophila* model systems of HD demonstrated that the potentially dysregulated huntingtin interaction partners influence polyQ-mediated protein misfolding and neurodegeneration. The neuron-specific protein CRMP1 e.g. is recruited to inclusion bodies with aggregated huntingtin protein in brains of HD transgenic mice and efficiently inhibits polyQ-mediated huntingtin exon 1 aggregation in cell free assays. Our results offer a new strategy for identifying perturbed, tissue-specific human PPIs and modulators of protein misfolding and aggregation (Bounab et al., 2009, submitted).

Development of interactome databases and novel quality standards for systematic protein interaction studies

Human protein interaction maps have become important tools of biomedical research for the elucidation of molecular mechanisms and the identification of new modulators of disease processes. We developed a comprehensive interactome database termed Unified Human Interactome (UniHI). It provides researchers with a comprehensive integrated platform to query and access human PPI data. Since its first release, UniHI has considerably increased in size. The latest update of UniHI includes over 250,000 interactions between ~23,000 unique proteins collected from 14 major sources. However, this wealth of data also poses new challenges for researchers due to the size and complexity of interaction networks retrieved from the database. We therefore developed several new tools to query, analyze and visualize human PPI networks. Most importantly, UniHI now allows the construction of tissue-specific interaction networks and focused searches of canonical pathways. This will enable researchers to target their analysis and to prioritize candidate proteins for follow-up studies. UniHI 4 can be accessed at http://www.mdc-berlin.de/unihi (Chaurasisa et al., 2008).

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Several attempts have been made to systematically map PPI networks. However, it remains difficult to assess the quality and coverage of existing data sets. In collaboration with the research group of Prof. Marc Vidal from Harvard Medical School, Boston, we have developed an approach to identify the most suitable quality parameters of currently available human interactome maps. We found that high-throughput yeast two-hybrid (HT-Y2H) screening yields more accurate interaction data for human proteins than non-systematic studies of small numbers of individual interactions. This suggests that HT-Y2H screening is a powerful method to map a significant portion of the human interactome. We estimated that the human interactome contains ~130,000 binary interactions, most of which remain to be mapped by systematic screenings. High quality data and accurate estimates of interaction numbers are crucial to establish the magnitude of the task of comprehensively mapping the human interactome (Venkatesan et al., 2009).

Constructing directed protein interaction networks for activated EGF/Erk signaling

Epidermal growth factor (EGF) signaling through extracellular-signal regulated kinases (Erks) is a complex process involving a series of protein-protein interactions (PPIs) that propagate information from the plasma membrane to transcription factors. To obtain a global view of EGF/Erk signaling and to predict potential modulators, we created a network connecting 1126 proteins via 2626 PPIs using automated yeast two-hybrid (Y2H) interaction mating. From this interaction map, a network of activated signaling was generated using a naïve Bayesian classifier, in which information on shortest PPI paths from membrane receptors to transcription factors was exploited to predict input/output relationships between interacting proteins. Analysis of the resulting network model revealed regulatory motifs typical for information processing systems. Moreover, it allowed predictions of potential modulators of EGF/Erk signaling, which were validated in mammalian cell-based assays. This generic experimental and computational approach provides a framework for elucidating causal connections between proteins and facilitates the identification of factors modulating the flow of information in signaling networks (Vinayagam et al., 2009, submitted).

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Ehrnhoefer, DE, Bieschke, J, Boeddrich, A, Herbst, M, Masino, L, Lurz, R, Engemann, S, Pastore, A, Wanker, EE. (2008) Redirecting aggregation pathways: small molecule-mediated conversion of amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nat Struct Mol Biol. 15(6), 558-66.

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Aging-related Protein Misfolding and Detoxification Mechanisms

The misfolding of endogenous protein or peptide fragments to form cytotoxic deposits made from fibrillar protein aggregates characterizes amyloid diseases. The misfolded polypeptides, which are believed to be the root cause of the pathologies, are specific for each disease, such as amyloid β (Aβ) in Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD) or fragments of the huntingtin protein in Huntigton's disease (HD). The specific nature and mechanism of the cytotoxicity are yet unknown. However, a wealth of evidence points to smaller oligomeric aggregates rather than large fibrils bein the crucial species.

Both AD and PD mostly occur sporadically without known genetic components. They have the patient's age as the single most important risk factor. Yet, little is known how aging influences them. Our lead hypothesis states that toxic protein aggregates result from an imbalance in the dynamic equilibrium between aggregation and clearance of misfolded proteins rather than from a slow stochastic process, as has previously been assumed.

The mechanistic details of protein misfolding, the autocatalytic replication of misfolded protein aggregates, and possible detoxifying mechanisms are the major points of our research (Fig. A). We found that misfolded Aβ aggregates are detoxified by two opposing activities under the control of two interconnected aging-related pathways, the Insulin/IGF-receptor pathway on one hand and the stress response / heat shock reponse pathways controlled by HSF-1 on the other hand (Fig B). The primary detoxification pathway appears to involve disassembly of misfolded aggregates, a secondary pathway the induced aggregation of small Aβ aggregates into larger, less-toxic structures.

Endogenous Modifiers of Amyloid Formation

We aim to identify key components of the cellular detoxification machinery and study the influence of endogenous proteins on aggregate assembly and disassembly using several screening based methods:

a. Cellular aggregation assays and siRNA interference

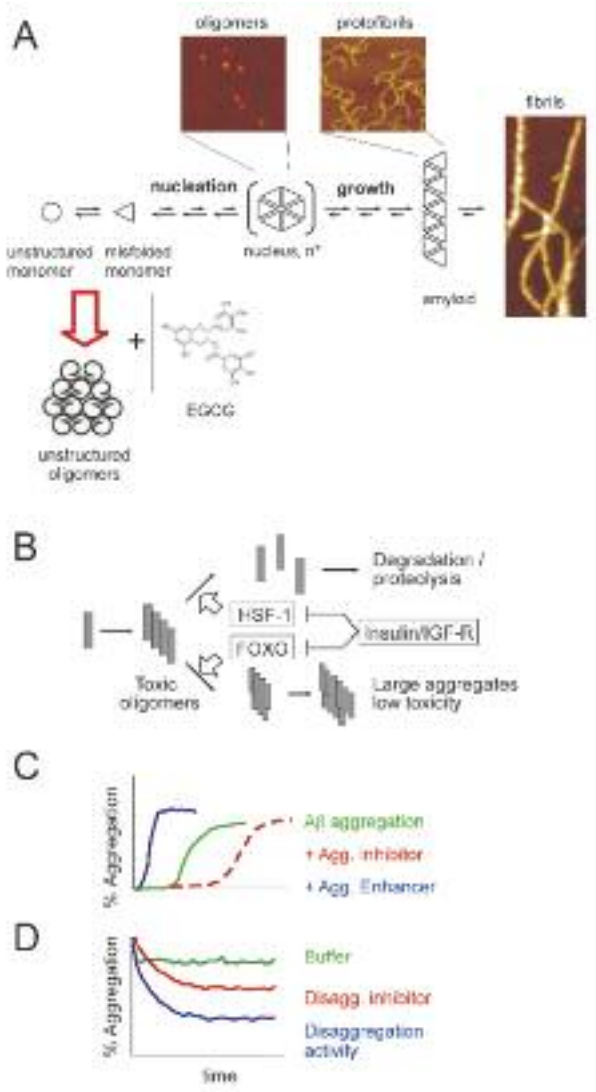
Katja Welsch

Aggregation-prone fragments of huntingtin are expressed in mammalian cells (N2A, PC12). Target genes from a library of proteins that are related to protein folding or to the aging pathways are simultaneously downregulated by siRNA during aggregation or after the completion of aggregation to assess their influence on amyloid formation and removal by the cells.

b. Genome-wide functional screening for amyloid modulators

Ralf Friedrich, Maliha Shah

Proteins are recombinantly expressed from a genomic human protein library (ca. 14.000 constructs) and added to *in vitro* aggregation assays and disaggregation assays (Fig C, D) using the Aβ peptides and the α-synuclein protein. Aggregation and disaggregation kinetics are used to identify proteins that influence aggregation or that have a disaggregating activity and



(A) Amyloid formation cascade. Unfolded or natively folded protein partially misfolds and associates in a multi-step process via oligomer and protofibril formation to form amyloid fibrils. EGCG redirects unfolded monomer into benign stable aggregates. (B) Schematic representation of detoxifying machinery controlled by the insulin signaling pathway. (C,D) Aggregation and disaggregation assays can be used to identify aggregation inhibitors and promoters in vitro *and from mammalian cells.*

hit proteins are being validated in mammalian cell culture experiments.

Amyloid Aggregate-Toxicity Relationship in Aβ and tau-Protein

Heike Wobst

Using model substances, such as EGCG and lipid oxidation products, as well as specific oligomer formation protocols, we create fibrillar and oligomeric species of Aβ peptides and tau-protein fragments that are either on-pathway or off-pathway to amyloid formation (Fig. A). We aim to understand the relationship between aggregation state and cytotoxicity of these species and test their capacity to inducue homologous and heterologous amyloid formation.

To that end we are, on one hand, focussing on detailed mechanistic studies using biophysical and biochemical assays, fluorescence techniques and atomic force microscopy. On the other hand we are correlating these results with aggregate uptake and toxicity assays in mammalian cell culture, to which aggregate sub-populations are added.

Mechanisms of Anti-Amyloid Drug Action

J. Bieschke, G. Grelle; collaboration with E. Wanker

The flavonoid (-)-epigallocatechin-gallate (EGCG) from green tea and related substances were found to be potent inhibitors of amyloid formation for a variety of polypeptides such as Aβ, α-synuclein and huntingtin. We found that EGCG prevents amyloid formation by a unique mechanism. It stimulates the production of off-pathway oligomers early in the aggregation process and thus diverts the misfolding process away form the toxic species (Fig. 1A). These substances can thus be used to probe the mechanism of amyloid formation and toxicity.

Selected Publications

Ehrnhoefer DE*, Bieschke J*, Boeddrich A, Herbst M, Masino L, Lurz R, Engemann S, Pastore A, Wanker EE. (2008). EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. Nature Struct Mol Biol. 15, 558-66.

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Berlin Institute of Medical Systems Biology (BIMSB)

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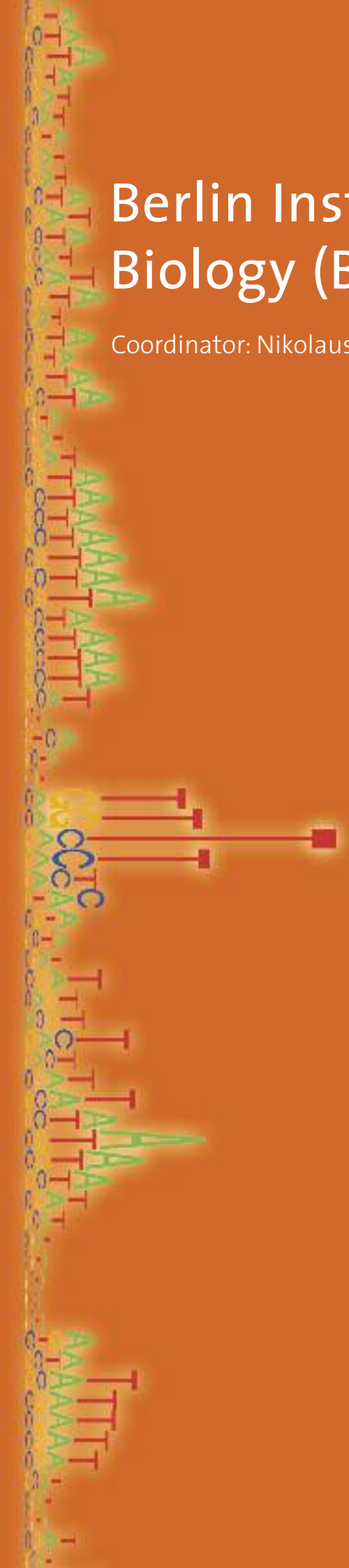

Berlin Institute for Medical Systems Biology, BIMSB

The MDC has launched a major expansion of its current scientific activities in the area of systems biology: The Berlin Institute for Medical Systems Biology. The aim of this initiative is to consolidate current scientific trends such as increasing interdisciplinarity, and the availability of huge amounts of data from new types of experiments and high-throughput genomic platforms. This information needs to be integrated into a systematic and quantitative understanding of all scales of life. The BIMSB is creating a research environment for excellent scientific groups with interdisciplinary and systems biology approaches at the MDC.

The Berlin Institute for Medical Systems Biology will closely collaborate with research institutions and universities in Berlin, and a new research building is planned to be constructed in Berlin-Mitte, most likely at the 'Campus Nord' of the Humboldt University. Promising talks have already been held between the MDC, HU-Berlin and the Berlin Senate.

The Federal Ministry of Education and Research (BMBF) has allocated 7.5 million euros for the period of 2008-2010 and the Senate of Berlin is complementing this with up to 4.4 million euros. During this period young research groups and an international exchange program will start their research and activities in Berlin-Buch. Major investments in high-end technologies will enable the BIMSB to set up state-of-the-art scientific technology platforms for genomics, proteomics, metabolomics and bioinformatics. The funding for the BIMSB was announced on May 5th 2008 by Germany's Education and Research Minister Annette Schavan as part of the BMBF initiative "Advanced Research and Innovation in the New States" (Spitzenforschung und Innovation in den neuen Ländern). During the initial phase, the first junior groups have been recruited and an international exchange program has been started with the Center for Functional Genomics of New York University (NYU), USA. This has been accompanied by major investments in technology platforms in the areas of genomics, proteomics, metabolomics and bioinformatics, which are used by MDC and external groups.

The scientific mission

Basic and biomedical research has provided a wealth of information about the role of individual genes in various diseases. Despite these discoveries and the progress made, it has been extremely challenging using this knowledge to understand disease and design and improve treatments. It is becoming increasingly clear that disease processes involve complex interactions between hundreds of genes, proteins and metabolites. Especially the most prevalent and devastating diseases such as cancer, cardiovascular disease, dia-

Mit dem zunächst auf Projektbasis operierenden Berliner Institut für Medizinische Systembiologie (BIMSB) und dem geplanten Ausbau als integraler Bestandteil des MDC hat das MDC eine bedeutende Entwicklung in der biomedizinischen Forschung, das Gebiet der Systembiologie, aufgegriffen. Das Ziel ist die Konsolidierung der gegenwärtig wissenschaftlichen Trends zu verstärkter Interdisziplinarität, der rasanten Genierung von sehr großen Datenmengen durch die immer stärkere Ausnutzung und Weiterentwicklung modernster Hochdurchsatz-Technologien und anderer Technologien, wie z. B. modernster Imaging-Verfahren. Daraus ergibt sich die Notwendigkeit der Integration dieser Vielzahl an Informationen, um ein systematisches, qualitatives und quantitatives Verständnis der Beziehungen innerhalb und zwischen den verschiedenen Ebenen biologischer Organisation, von der Funktion einzelner Gene bis hin zu komplexen Wechselwirkungen in Zellen, Organen und Organismen, zu erreichen. Im BIMSB werden exzellente wissenschaftliche Forschungsgruppen mit interdisziplinärer und systembiologischer Ausrichtung zusammengeführt. Durch die enge Vernetzung innerhalb des MDC mit den bestehenden MDC-Gruppen können vorhandene Stärken mit neuesten Entwicklungen optimal verbunden und ausgenutzt werden. Bereits jetzt schon unterstreichen erste Ergebnisse erfolgreicher Zusammenarbeit, die auch in diesem Bericht zu finden sind, die Berechtigung und Richtigkeit des eingeschlagenen Wegs. Darüber hinaus wird die enge Kooperation des MDC mit den anderen Forschungseinrichtungen in Berlin und insbesondere den Berliner Universitäten über das BIMSB vertieft und stark ausgebaut. Daher hat sich das MDC entschlossen, das neue Forschungsgebäude zentral in der Mitte Berlins, voraussichtlich auf dem Campus-Nord der Humboldt-Universität zu Berlin, zu errichten. Erste vielversprechende Gespräche zwischen MDC, HU-Berlin und dem Berliner Senat wurden bereits geführt.

Für die Startphase des BIMSB hat das Bundesministerium für Bildung und Forschung (BMBF) für den Zeitraum von 2008 bis 2010 7,5 Millionen y bereitgestellt. Um 4,4 Millionen y hat der Senat von Berlin diese Mittel ergänzt. Die Bereitstellung der Mittel für das BIMSB als Teil der BMBF-Initiative „Spitzenforschung und Innovation in den neuen Ländern" wurde am 5. Mai 2008 durch die Ministerin für Bildung und Forschung, Dr. Annette Schavan, verkündet. Während dieser Startphase wurden die ersten Nachwuchsforschungsgruppen rekrutiert und ein internationales Austauschprogramm mit dem Zentrum für Funktionelle Genomik der New York University (NYU), USA, gestartet. Durch große Investitionen in Technologieplattformen im Bereich Genomik, Proteomik, Metabolomik und Bioinformatik wurde eine ausgezeichnete infrastrukturelle Basis geschaffen, die von den Gruppen des MDC und externen Gruppen genutzt wird.

betes, metabolic diseases and neurodegenerative disorders are multifactorial. Such complex systems can in most cases only be understood by quantitative models that predict the interactions and functions of numerous components. Never before has it been possible to collect complete data from a minimal amount of biological data about the condition of a cell, organ, or organism at the levels of the genome, proteome, and metabolome. This information can only be understood with the help of mathematical models. Thus Systems Biology is by nature highly interdisciplinary and combines molecular biology, biochemistry, mathematics, physics and engineering.

The importance of Systems Biology in biomedical research is particularly apparent in the field of post-transcriptional gene regulation and RNA biology (Special issue, *Cell* 2009). Additionally many new classes of non-coding and small RNAs (such as microRNAs, piRNAs, and many others) have been recently discovered and have been shown to act in the regulatory networks in all multicellular organisms. Furthermore, the human genome encodes hundreds of proteins with RNA binding domains. These proteins function in regulating mRNA localization, mRNA turnover, protein synthesis and other crucial steps that control gene expression. Small RNAs and RNA binding proteins have been shown to be in many ways relevant players in regulation of phenotypes in health and disease and have therefore a huge potential for medical applications. In short, the grand challenge and overall scientific mission for the BIMSB is to decipher the 'post-transcriptional regulatory code' and to directly integrate it with other major cellular regulatory mechanisms, in particular transcriptional regulatory circuits, signal transduction pathways, protein-protein interactions networks, and post-translational modifications.

Advanced technologies like next generation sequencing and mass-spectrometry, combined with imaging approaches and biochemical methods, will allow genome-wide quantitative analysis of regulatory mechanisms and resolution of RNA function on many different levels.

BIMSB research groups, scientific platforms and the international PhD exchange program

Young scientists leading independent research groups and scientific platforms in genomics, proteomics and quantitative biology have been appointed at the BIMSB. A few examples of the ongoing work reveal how their research can complement and expand on the MDC's overall mission of "molecular medicine."

Das wissenschaftliche Programm

In der biomedizinischen Grundlagenforschung konnten in den vergangenen Jahrzehnten eine Fülle an Informationen über die Rolle einzelner Gene bei Erkrankungen gewonnen werden. Trotz dieser Entdeckungen und dem damit einhergehenden Erkenntnisfortschritt ist es immer noch ein großes Problem, dieses Wissen für das Verständnis von komplexen Erkrankungen und damit für die Entwicklung neuer und die Verbesserung bestehender Therapien zu nutzen. Es hat sich klar gezeigt, dass Krankheitsprozesse aus der Wechselwirkung von Hunderten von Genen, Proteinen und Stoffwechselprodukten entstehen. Besonders die häufigsten und bedrohlichsten Erkrankungen wie Krebs, kardiovaskuläre Erkrankungen, Diabetes, metabolische und neurodegenerative Störungen sind multifaktoriell bedingt. Solche komplexen Systeme können in den meisten Fällen nur mit Hilfe mathematischer Modelle verstanden werden, die die Interaktionen und Funktionen der zahlreichen Komponenten integrieren und damit eine Vorhersage der Auswirkungen auf das Gesamtsystem ermöglichen. Systembiologie ist hoch interdisziplinär und umfasst neben Molekularbiologie und Biochemie vor allem Mathematik, Physik und Ingenieurwissenschaften. Nie zuvor war es möglich, den gesamten Datensatz zum „Zustand" einer Zelle/Organ/Organismus zu einem bestimmten Zeitpunkt mit einem Minimum an biologischem Material auf Genom-, Transkriptom-, Protein- und Metabolomebene zu erhalten. Die daraus resultierenden großen Datenmengen sind ohne mathematisches Verständnis nicht zu bewältigen. Letztendlich schafft dieses Herangehen jedoch völlig neue Möglichkeiten für eine systematische Analyse verschiedener Zustände und damit ein neues Verständnis der Mechanismen komplexer Systeme.

Die Bedeutung einer systembiologischen Herangehensweise lässt sich besonders anhand der auf diese Weise gewonnenen Erkenntnisse auf dem Gebiet der post-transkriptionalen Genregulation und RNS-Biologie (Spezialausgabe von Cell, 2009) illustrieren. Darüber hinaus wurden in letzter Zeit neue Klassen nicht-kodierender RNS (microRNS, piRNS usw.) entdeckt, die die regulatorischen Netzwerke aller multizellulären Organismen beeinflussen. Es konnte nachgewiesen werden, dass kleine RNS-Moleküle und RNS-bindende Proteine wichtige Regulationsfaktoren für die Ausbildung verschiedener Phänotypen in normalen und kranken Organismen sind. Das menschliche Genom kodiert für Hunderte von Proteinen mit RNS-Bindungsdomänen. Diese Proteine regulieren die Synthese und die Lokalisierung der entsprechenden Boten-RNS, die Proteinsynthese selbst und andere wichtige Schritte der Genexpression. Diese Befunde eröffnen völlig neue Möglichkeiten für die klinische Anwendung. Kurz gesagt, die wissenschaftliche Mission des BIMSB ist es, den „post-translationalen Code" zu entziffern und ihn mit anderen zellulären Regulationsebenen, insbesondere der transkriptionalen regulatorischen Kreisläufe, den Signalketten und Signaltransduktionswegen, Pro-

Markus Landthaler is an outstanding scientist in the field of post-transcriptional regulation and RNA binding proteins. His research aims for a systems level understanding of post-transcriptional regulation and a transcriptome-wide high-resolution map of RNA-protein interaction. Markus Landthaler's detailed research profile appears on page 62.

The Genomics Platform is lead by Wei Chen, an expert in next-generation sequencing technologies and their application to systems wide approaches in genomic research. Christoph Dieterich heads the Bioinformatics Platform and will perform and support the computational side of genome-wide analysis and bioinformatics. The third platform, headed by Stefan Kempa, will enable to MDC and BIMSB research groups to analyse the proteome and metabolome by mass-spectromentry in a high-throughput format. The integration of the technologies of all scientific platforms will support systems-wide understanding of complex regulatory mechanisms.

Another focus of the BIMSB is the international and interdisciplinary training of young scientists, especially PhD students. A first step has been to develop a meaningful exchange program jointly operated by the MDC and New York University (NYU) in the United States. Other international partnerships and scientific exchange programs are being negotiated, e.g. with Kyoto Medical School, Japan, and further systems biology institutes in the USA. Within the MDC-NYU PhD exchange program students will carry out collaborative projects between the Center for Functional Genomics in New York and the BIMSB in Berlin and may spend up to 50% of their time in either location. They are co-mentored by NYU and MDC faculty and can participate in projects and classes at both institutions. In 2009 this collaboration has already led to important publications and many others are in the pipeline. Additionally, the BIMSB is very active in coordinating scientific workshops for communication and collaborative efforts with local, national and international partners.

Partners

By definition, systems biology must be open to the integration of a wide range of expertise. The BIMSB's strategic concept has been developed in collaboration with virtually all local universities and scientific institutions in Berlin as well as other partners in Germany. Several projects funded by the BMBF, Helmholtz Association, the DFG and other sources are ongoing between BIMSB investigators and collaborators throughout Berlin.

tein-Protein-Interaktions-Netzwerken und posttranslationalen Modifikationen zu verzahnen.

Modernste Sequenzierungs- und Massenspektrometrietechnologien in Verbindung mit modernen bildgebenden Verfahren und biochemischen Methoden werden eine genomweite qualitative und quantitative Analyse der Regulationsmechanismen, insbesondere der Aufklärung der RNS-Funktion auf den verschiedenen Hierarchieebenen, ermöglichen.

BIMSB Forschergruppen, wissenschaftliche Plattformen und das Internationale PhD-Austausch-Programm

Innerhalb der erst kurzen Laufzeit konnten bereits herausragende junge Wissenschaftler als Leiter unabhängiger Forschungsgruppen und wissenschaftlicher Plattformen im Bereich Genomik, Proteomik und quantitative Biologie rekrutiert werden. Die hier im Bericht aufgeführten Beispiele der laufenden Forschung zeigen, wie ihre Arbeiten die Gesamtmission des MDC in der „Molekularen Medizin" ergänzt und erweitert.

Markus Landthaler ist ein hervorragender Wissenschaftler auf dem Feld der post-transkriptionalen Regulation und der RNS-bindenden Proteine. Er arbeitet an einem systemischen Verständnis der post-transkriptionalen Regulation und an einer transkriptom-weiten hochauflösenden Karte von RNS-Protein-Interaktionen. Sein Forschungsprofil wird im vorderen Teil des Berichtes detailliert dargestellt (Seite 62).

Die Genomik-Plattform wird von Wei Chen geleitet, einem Experten für die neue Generation von Sequenziertechnologien und ihre Anwendung in der systemweiten Genomforschung. Christoph Dieterich leitet die Bioinformatik-Plattform und wird die algorithmisch-bionformatische Seite der genomweiten Analysen abdecken. Die dritte Plattform, geleitet von Stefan Kempa, wird den MDC- und BIMSB-Gruppen ermöglichen, das Proteom und das Metabolom mit Hilfe von Massenspektrometrie im Hochdurchsatzformat zu analysieren. Die Integration dieser methodischen Plattformen wird ein systemweites Verständnis komplexer regulatorischer Mechanismen unterstützen.

Ein weiterer Schwerpunkt des BIMSB ist eine international ausgerichtete interdisziplinäre Ausbildung junger Wissenschaftler, speziell von Doktoranden. Ein erster Schritt ist die Etablierung des Austauschprogramms, das gemeinsam vom MDC und von der New York University (NYU) in den USA organisiert wird und in 2009 gestartet wurde. Weitere internationale Partnerschaften sind in Vorbereitung, z.B. mit der Kyoto Medical School in Japan und Systembiologie-Instituten in den USA. Im MDC-NYU-Programm werden gemeinsame Projekte zwischen dem Zentrum für Funktionelle Genomik in New York und dem BIMSB in Berlin durchgeführt, so dass die Teilnehmer bis zu 50% der Zeit in der jeweils anderen Institution verbringen können. Dies wird von den beiden Fakultäten gemeinsam begleitet und beinhaltet die Teilnahme an Seminaren in beiden Institutionen. Diese Kooperation hat in 2009 bereits eine Publikation in ‚Nature Methods' ergeben. Insgesamt wird das

A number of MDC investigators have substantially contributed to the scientific concept and are involved through collaborative projects; these include Jana Wolf, Matthias Selbach, Norbert Hübner, Erich Wanker, Miguel Andrade-Navarro, Martin Falke, and investigators from the Leibniz Institute for Molecular Pharmacology (FMP).

The BIMSB investigators have additionally established industry collaborations for joint projects and technology development (i.e. with Applied Biosystems, Qiagen and Roche).

International collaborations are initiated or established with the NIH-supported centers for systems biology, the New York University and the Rockefeller University. Additionally, the European Molecular Biology Laboratory and EU-funded networks and ESF policy agendas have greeted, supported and integrated the BIMSB in discussions on future systems and RNA biology efforts on a national and European level.

Future perspectives

The BIMSB will become an institutional branch of the MDC in Berlin-Mitte, a location that will more closely interconnect the MDC with experimental and theoretical institutions in Berlin including universities, the Charité University hospital, research institutions and the DFG Research Center Matheon and other non-university research institutes. This interdisciplinary interface between universities and research institutions will strengthen the scientific profile of Berlin.

The structure and size of the new building in Berlin-Mitte will offer research space for up to 25 research groups plus 5 technology platforms, ample space for visiting scientists and extensive workshop and conference facilities.

In 2009, the performance of the existing BIMSB activities as well as the overall scientific concept of the BIMSB were reviewed by an international panel of experts in systems biology, and RNA biology. The panel strongly supported the MDC in proceeding on this endeavour and expanding and establishing the BIMSB, with this scientific mission, the proposed size, funding, and the location in Berlin-Mitte.

BIMSB durch die Organisation wissenschaftlicher Workshops und anderer Veranstaltungen den Erfahrungsaustausch und die Zusammenarbeit mit regionalen, nationalen und internationalen Partnern fördern.

Systembiologie steht für die Integration eines breiten Wissens aus unterschiedlichsten Fachgebieten. Das strategische Konzept des BIMSB wurde in enger Abstimmung mit nahezu allen universitären und außeruniversitären Forschungseinrichtungen im Land Berlin und anderen Partnern in Deutschland erarbeitet. Die Basis für die weitere enge Zusammenarbeit bilden bereits jetzt schon mehrere vom BMBF, der DFG und aus anderen Quellen finanzierte Kooperationsprojekte.

Viele MDC-Wissenschaftler haben entscheidend zu diesen neuen Entwicklungen und zum wissenschaftlichen Konzept beigetragen und sind in verschiedene gemeinsame wissenschaftliche Projekte eingebunden, unter ihnen Jana Wolf, Matthias Selbach, Norbert Hübner, Erich Wanker, Miguel Andrade-Navarro, Martin Falcke und weitere Kollegen aus dem Leibniz-Institut für Molekulare Pharmakologie (FMP). Darüber hinaus haben die Wissenschaftler des BIMSB auch gemeinsame Kooperationsprojekte mit der Industrie zur Technologie-Entwicklung (z.B. mit Applied Biosystems, Qiagen, Roche) gestartet. Internationale Kooperation in größerem Umfang wurden mit den NIH-geförderten Zentren für Systembiologie an der New York University und der Rockefeller University initiiert. Das European Molecular Biology Laboratory (EMBL), EU-finanzierte Forschungsnetzwerke auf dem Gebiet Systembiologie und verschiedene ESF-Gremien haben das MDC zum geplanten substantiellen Ausbau der Systembiologie im Rahmen des BIMSB beglückwünscht.

Zukunftsperspektiven

Das BIMSB als Teil des MDC wird durch seinen Standort in Berlin-Mitte das MDC insgesamt noch enger mit den experimentellen und theoretischen Institutionen der Universitäten, der Charité-Kliniken, dem DFG-Forschungszentrum Matheon und weiteren außeruniversitären Forschungsinstituten verbinden. Es wird als interdisziplinäre Schnittstelle zwischen Universitäten und außeruniversitären Institutionen das wissenschaftliche Profil Berlins stärken. Das neue Gebäude in Berlin-Mitte wird Platz für bis zu 25 Forschergruppen und 5 Technologieplattformen bieten, ergänzt durch Seminar- und Konferenzräume. Sowohl die bisherige Leistung als auch das wissenschaftliche Zukunftskonzept wurden im Frühjahr 2009 einer umfassenden Begutachtung durch ein internationales Expertenteam unterzogen. Die Gutachterkommission setzte sich aus weltweit anerkannten Wissenschaftlern auf dem Gebiet der RNS-Biologie und der Systembiologie zusammen. Die Gutachterkommission hat diese bisherigen Aktivitäten und das wissenschaftliche Konzept des MDC zur Errichtung des BIMSB begeistert begrüßt und nachhaltig unterstützt. Sowohl Größe und Umfang des geplanten Instituts als auch der vorgesehene Standort in Berlin-Mitte wurden als angemessen und notwendig erachtet.

Wei Chen

Structure of the Group

Group Leader
Dr. Wei Chen

Scientists
Dr. Yuhui Hu
Dr. Andreas Doering

Graduate Students
Na Li
Hui Kang
Wei Sun
Yongbo Wang
Raghu Bhushan (till June 2009)

Novel sequencing technology, miRNA regulation and human molecular genetics

The recent introduction of massive parallel sequencing technology has revolutionized genomic research. These so-called next generation sequencing platforms, such as Roche/454, Illumina/solexa and ABI/Solid system can sequence DNA orders of magnitude faster and at much lower cost than conventional Sanger method. With their incredible sequencing capacity, my lab has been focused on developing and implementing various genomic assays based on this new generation of sequencers. Apart from offering services to the institute as a core facility provider, we are now applying the assays in studying transcriptional and posttranscriptional regulation of miRNA genes, as well as identifying genetic factors underlying human diseases.

Transcriptional and posttranscriptional regulation of miRNA genes

Yuhui Hu, Andreas Doering, Na Li, Hui Kang, Wei Sun

MiRNAs are small non-coding RNAs that control the expression of target genes at the posttranscriptional level. Recently, more and more miRNAs have been implicated in a variety of biological processes. Whereas much attention has been focus on finding the target genes regulated by miRNAs, little is known about the system which regulates miRNA expression. One major focus of my lab is to study transcription and posttranscriptional regulation of miRNA genes. In the study of transcriptional regulation, we are involved in genome wide discovery of miRNA promoters in pre-B cells using ChIP-seq, mRNA-seq and small RNA sequencing methods.

It has been demonstrated that the Drosha or Dicer processing of individual miRNA can be regulated. Though, it is yet not known that how generalized the phenomena are. We are therefore interested in studying the posttranscriptional regulation of miRNAs, especially regulation of Dicer processing at the genomic level by genome-wide profiling of miRNA precursor (pre-miRNA) and mature miRNA from the same sample and comparing their relative abundance across different samples. Currently, we are developing a novel assay to efficiently profile pre-miRNA based on new sequencing technology.

Characterisation of breakpoints in disease-associated balanced chromosome rearrangements

Yuhui Hu, Andreas Doering

Balanced chromosome rearrangements (BCRs) can cause genetic diseases by disrupting or inactivating specific genes, and the characterization of breakpoints in disease-associated BCRs has been instrumental in the molecular elucidation of a wide variety of genetic disorders. However, mapping chromosome breakpoints using traditional methods is rather laborious and time-consuming. In addition, the resolution is often insufficient to unequivocally identify the disrupted gene. To

Technical Assistants
Claudia Langnick
Mirjam Feldkamp
Regine Schwartz

Start of group: January 2009

overcome these limitations, we have developed novel methods based on new sequencing technology to characterize the breakpoints in an efficient manner. We are now implementing our method in large-scale breakpoint mapping and gene finding.

De novo transcriptome sequencing using 454 pyrosequencing

Yongbo Wang

New sequencing technologies are not only robust tools for the investigation of transcriptome in model organisms, but also manifest great potential in studying non-model organisms. To facilitate comprehensive transcriptome characterization, we are developing methods for transcriptome sequencing using 454 pyrosequencing. With long read length and high accuracy, it is particularly suitable for de novo sequence assembly. To be fit for 454 sequencing, our methods consist of steps to remove poly A+ tails and cDNA library normalization. The sequencing data would provide a comprehensive reference resource for further functional studies.

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*shared corresponding author

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Friedländer MR, Chen W, Adamidi C, Maaskola J, Einspanier R, Knespel S, Rajewsky N. (2008). Discovering microRNAs from deep sequencing data using miRDeep Nat Biotechnol. 26(4), 407-415.

Chen W, Kalscheu V, Tzschach A, Menzel C, Ullmann R, Schulz M, Erdogan F, Li N, Kijas Z, Arkesteijn G, Pajares IL, Goetz-Sothmann M, Heinrich U, Rost I, Dufke A, Grasshoff U, Glaeser BG, Vingron M, Ropers HH.(2008) Mapping translocation breakpoints by next-generation sequencing. Genome Res. 18: 1143-1149

Stefan Kempa

Structure of the Group

Group Leader
Stefan Kempa

Scientists
Guido Mastrobuoni

Graduate Student
Matthias Pietzke

Undergraduate Student
Christin Hess

Technical Assistant
Julia Diesbach

Secretariat
Sabrina Deter

Start of group: April 2009

Integrative metabolomics and proteomics

Within the past decades biochemical data of single processes, metabolic and signaling pathways has been collected. Advances in technology led to improvements of sensitivity and resolution of bioanalytical techniques. These achievements build the basis of so-called 'genome wide' analyses.

High throughput techniques are the tool for such large scale "-omics" studies allowing the obtainment of a complete picture of a determinate cell state, concerning its metabolites, transcripts and proteins. For example two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-TOF-MS) is a promising technique to overcome limits of complex metabolome analysis using one dimensional GC-TOF-MS. However, single level study of a living organism (transcripts, proteins or metabolites) cannot give a complete understanding of the mechanism regulating biological functions. The integration of transcriptomics, proteomics and metabolomics data in the newly emerging field of System Biology, combined with existing knowledge, allows connecting biological processes which were treated as independent so far. In this context the aim of our group is to apply metabolomics and proteomics techniques for absolute quantification and analysis of turnover rates of proteins and metabolites using stable isotopes; in addition, the further development of workflows for data analysis and integrative strategies will be in the focus of our interest.

Functional analysis of cancer metabolism

Matthias Pietzke, Julia Diesbach, Markus Landthaler and Jana Wolf

Beside the enormous diversity of cancers, all tumor cells seem to share the same metabolic disorder. Already in 1924 the famous Nobel Prize biochemist Otto Warburg described an anaerobic type of metabolism performed by cancer tissues. This phenomenon, called Warburg effect, is still under investigation and the benefit for cancer cells from this metabolic behavior is still obscure; but it might stem from the onset of cancerogenesis. To unravel the regulatory mechanisms inducing this metabolic disorder we perform integrative transcriptomic, proteomic and metabolomic studies. These analyses will allow understanding the molecular processes leading to the observed metabolic alterations of cancer cells and could help to improve anti cancer therapies.

Planaria as model organism

Guido Mastrobuoni, Julia Diesbach, Sebastian Mackowiak and Catherine Adamini (Rajewsky Group)

The planarian *Schmidtea mediterranea* is a well-established model organism at BIMSB. These freshwater flatworms can regenerate all lost body tissue after amputation due to a population of pluripotent somatic stem cells called neoblasts that constitute up to 30 percent of the total organism cells.

In parallel to other studies carried at BIMSB, aimed to understand the role of small RNAs in the planarian development and regeneration of their germ cells, our group is involved in collaborative projects for the functional annotation of Planaria genome using proteomic data (figure 1).

Once a full genome has been assembled, the main challenge lies in its annotation, i.e., in identifying the protein-coding genes and other functional units that are encoded in the genome. Whole-genome annotation

has become a standardized data flow and initial sets of all encoded proteins can be generated in a computer-assisted and automated way.

The integration of metabolomic and proteomics technologies into the annotation process gives an experimental validation of in *silico* gene models as well as to improved accuracy of existing gene models.

BIMSB systems database

Guido Mastrobuoni, Matthias Pietzke and Christoph Dieterich

Modern "-omics" technologies like transcriptomics, proteomics and metabolomics are established at BIMSB. Those techniques generate large amounts of data and cover a wide range of biological processes. To analyze and store such large amounts of data from these technologies we are establishing the BIMSB systems database. The database will consist of several modules for data input and subsequent data analysis and comparison, thus it will connect the analytical platforms with computational biologists (see figure 2). It will help to structure and synchronize large datasets from multiple analytical platforms and will build up the basis for integrative analyses.

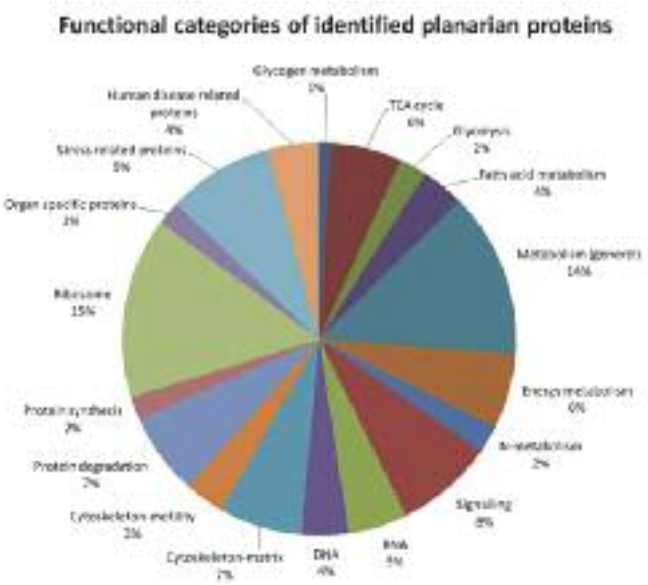

Figure 1. ***Functional categories of identified planarian proteins.*** *Total protein extract from whole worm was enzymatically digested and the resulting peptide mixture analyzed by LC-MS/MS on an Orbitrap mass spectrometer. The first analysis allowed the matching of around 35% of MS/MS peptide fragmentation spectra to protein sequences from a database of predicted gene products.*

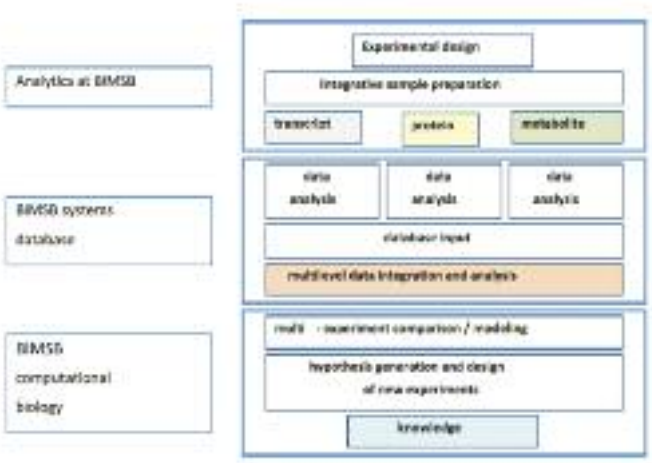

Figure 2. ***Scheme of BIMSB systems database.*** *The database consists of several modules from data input, analysis, storage and subsequent analyses. It will be a tool to analyze large datasets from different analytical platforms and will allow interspecies comparison on a quantitative level. The aim of the database is to connect the analytical platforms with the computational biologists at the BIMSB.*

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Christoph Dieterich

Structure of the Group

Group Leader
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Dominic Tölle

Graduate Students
Sebastian Fröhler
Zisong Chang

System administrator
Andreas Kuntzagk

Secretariat
Sabrina Deter

Start of group:
April 2009

Bioinformatics in Quantitative Biology

Bioinformatics is a highly dynamic discipline, which operates at the interface of life sciences, computer science and formal sciences. Currently, emerging technologies in nucleic acids sequencing, mass spectrometry and imaging revolutionize biology. For the first time, a holistic quantification of biological systems at the level of genomes, transcripts, proteins and metabolites is in reach. Bioinformatics supports this technology-driven transition of biology into a truly quantitative science.

We use existing and develop novel methods for high-throughput data acquisition, processing, model building and inference. Computational studies are backed up by experimental work within our group and within active collaborations.

Computational approaches to study eukaryotic gene regulation

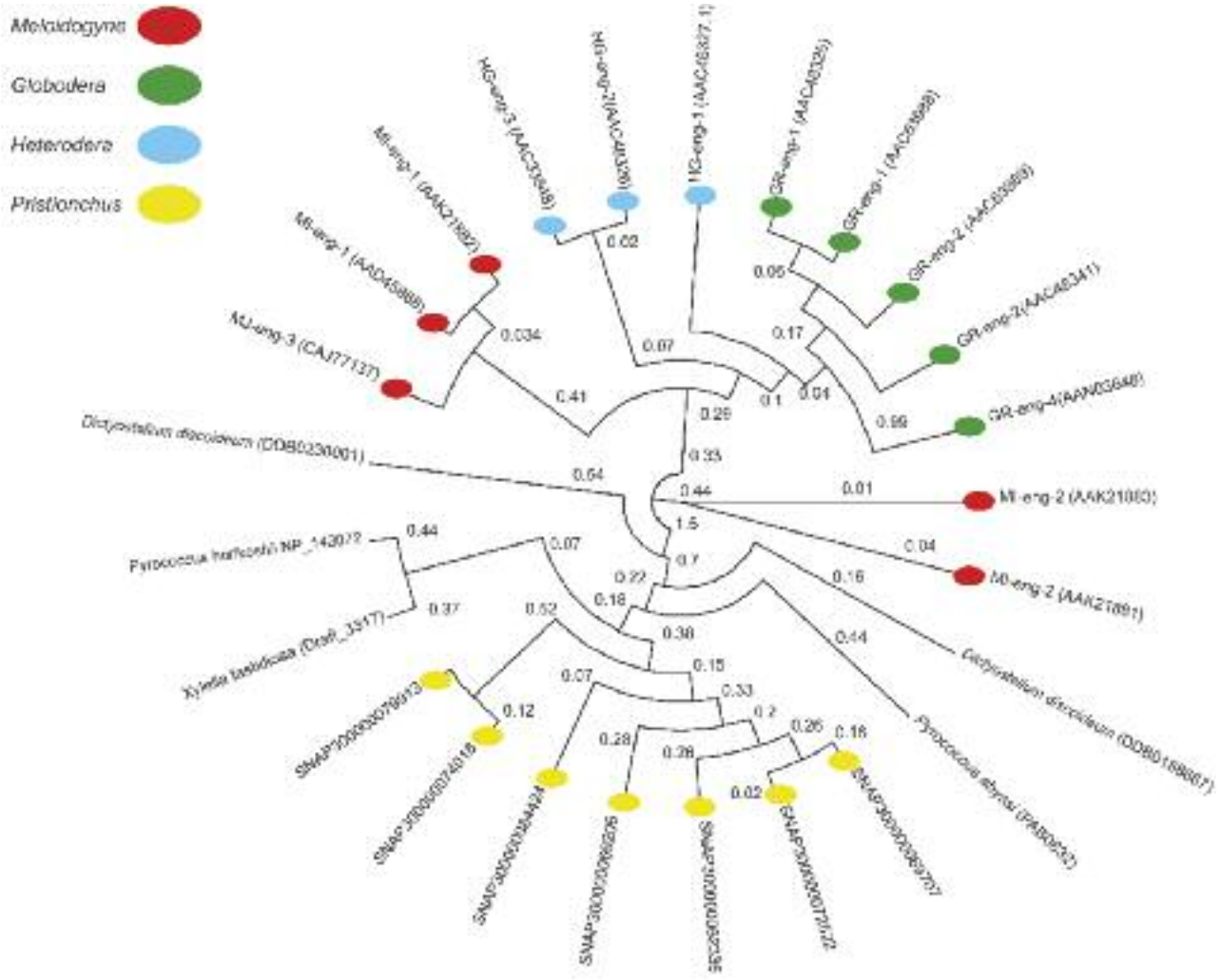
The expression of a gene is directly regulated by interactions of proteins and nucleic acids or by RNA-RNA interactions. We work on statistical methods for regulatory motif (e.g. binding site) and module (e.g. promoter elements) prediction. Lately, we explore the use of supervised sequence segmentation approaches to pinpoint enhancer regions (HM-SVMs). The evolution of binding sites is another important aspect, we pay attention to. Long-ranging interactions between different genomic loci are still enigmatic, yet are of tremendous importance to gene regulation. We support this important endeavor by an experimental design algorithm for the chromatin conformation capture (3C) technique.

Pristionchus – a model system in ecology

Pristionchus pacificus has been established as a satellite system to *Caenorhabditis elegans* over the last years. *P. pacificus* is an omnivorous nematode and lives in a necromenic association with scarab beetles, which is thought to constitute an evolutionary intermediate between free living nematodes and true parasites. To complement our biological knowledge, we sequenced the *P. pacificus* genome and found it to be enriched for gene families involved in stress response and metabolism of xenobiotics. Additionally, there are several instances of lateral gene transfer. For example, cellulase gene predictions were confirmed by experiment and shown to be integrated into the host biology by activity assays. Another set of predicted protein-coding genes did not show any similarity to the known protein universe and is currently being confirmed by transcriptome sequencing and mass spectrometry approaches.

Genome evolution – content, order and variation

Phylogenetic profiling of gene content may guide us in studying species adaptation to certain environments. Similarly, gene order is constrained by several partly unknown factors. We invented algorithms for identifying gene order conservation without assigning orthology a priori. We are able to look deep into the history of genome structure evolution since these approaches do not rely on a given whole-genome alignment. We are likewise interested in reconstructing the order of these events (in collaboration with Jian Ma, UCSC). Sequence variation (SNPs and Indels) is another layer of genome



Phylogeny of cellulase genes from P. pacificus and plant parasitic nematodes. The phylogeny of cellulases indicates that plant parasitic nematodes (green) and P. pacificus (red) have acquired cellulases independently by horizontal gene transfer. The circular phylogram and branch length estimates (expected number of amino acid substitutions) are based on a multiple alignment of the conserved region (70 amino acids) around the C-terminal active site. Only nematode cellulases with >10% sequence divergence are shown. Additionally, best non-nematode protein matches to P. pacificus cellulases are shown.

evolution. We detect sequence variation from high-throughput sequencing approaches and predict potential effects on the phenotype with expressive graphical models.

Selected Publications

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Dieterich, C.; Clifton, S. W.; Schuster, L. N.; Chinwalla, A.; Delehaunty, K.; Dinkelacker, I.; Fulton, L.; Fulton, R.; Godfrey, J.; Minx, P.; Mitreva, M.; Roeseler, W.; Tian, H.; Witte, H.; Yang, S.-P.; Wilson, R. K. & Sommer, R. J. (2008), 'The Pristionchus pacificus genome provides a unique perspective on nematode lifestyle and parasitism.', Nat Genet 40(10), 1193-1198.

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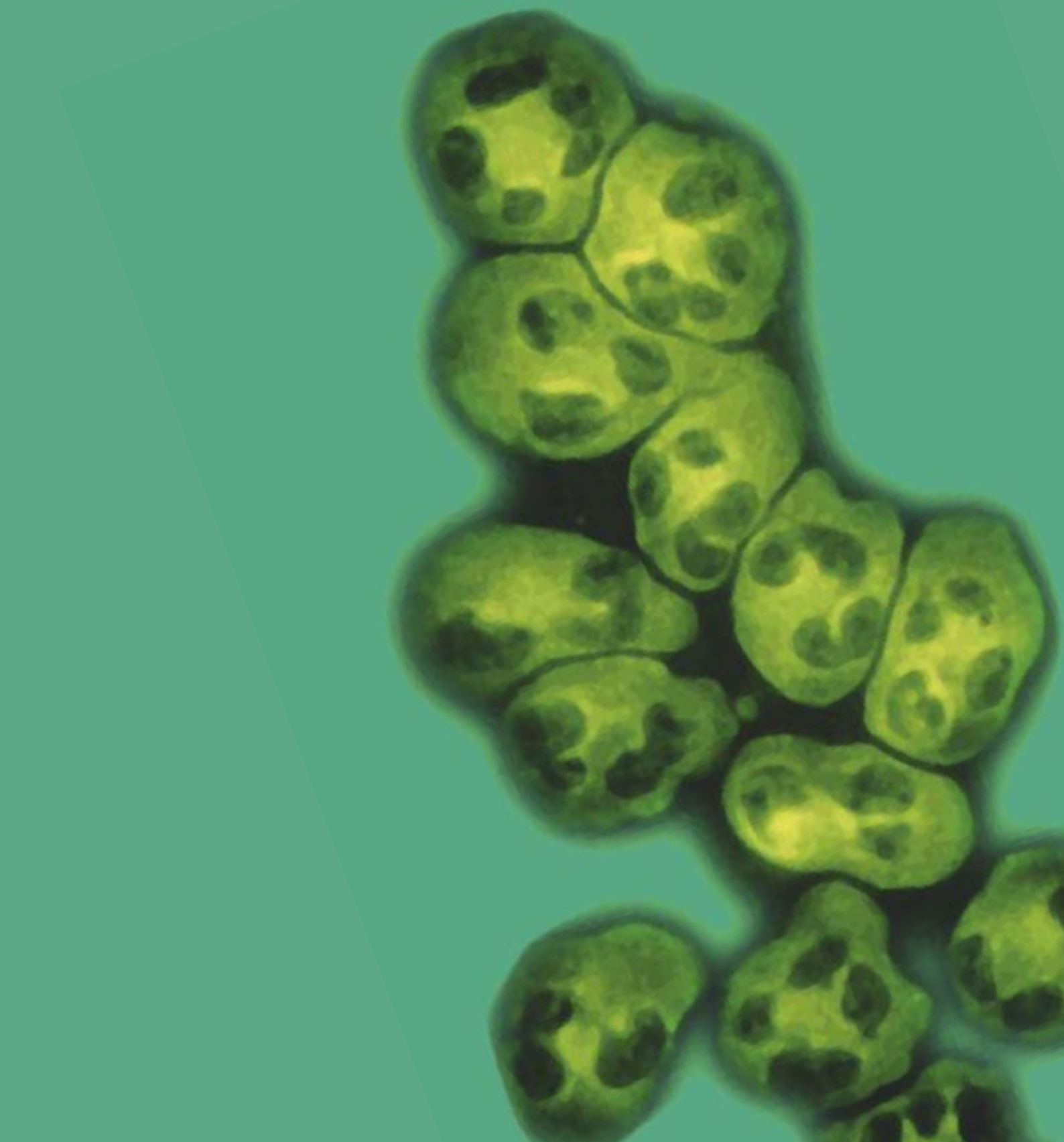
The Experimental and Clinical Research Center (ECRC)

Director: Friedrich Luft



Experimental and Clinical Research Center (ECRC)

Experimental and Clinical Research Center (ECRC)

The ECRC´s mission is to accelerate the translation of discoveries from fundamental research into new procedures for diagnoses, prevention, and therapies for the most common diseases that threaten our society. (The focus of the ECRC´s research is on cardiovascular and metabolic diseases, cancer, and neurological disease – corresponding to the research programs of the MDC). The ECRC is a joint institutional infrastructure dedicated to supporting collaborative research projects and patient-oriented research and was launched in 2007 by the MDC and the Charité.

The ECRC creates multiple links between basic researchers and clinicians, thus fostering the bidirectional transfer of knowledge between the two "worlds", which is considered a key factor for successful "translational" research.

The ECRC provides scientists from the Charité and MDC extensive opportunities for carrying out translational projects:

- Clinicians or basic researchers can apply for independent project groups to conduct a translational project within the ECRC. Funding is provided for three years (extension possible). The funding decision is based on a competitive evaluation involving external experts.
- Clinicians and researchers of the MDC can jointly submit proposals for collaborative projects which can be funded through the ECRC after a competitive evaluation (KKP program). The projects are conducted at the ECRC and/or the MDC.
- Within the ECRC, the MDC and Charité jointly sponsor a training program for clinicians (KAP, see below) in basic molecular biology, in which clinicians take a break from their duties and join one of the MDC's laboratories. This promotes long-term collaborations between the home and host.
- Clinicians direct out-patient clinics and carry out clinical studies within the ECRC;
- Clinicians and scientists have access to clinical research center (outpatients clinics, beds for clinical trials, see below) and technology platforms, (e.g. ultrahigh-field MR instruments, GMP facility, biobanking).

Das ECRC hat zum Ziel, die Übertragung von Erkenntnissen der Grundlagenforschung in neue Verfahren zur Vorbeugung, Diagnose und Therapie von Krankheiten unserer Gesellschaft zu beschleunigen. Der Schwerpunkt der Forschung im ECRC liegt auf Herz-Kreislauf- und Stoffwechselkrankheiten, Krebs- und neurologischen Erkrankungen und entspricht den Forschungsprogrammen des MDC.

Seit seiner Gründung im Jahr 2007 bildet das ECRC eine gemeinsame institutionelle Infrastruktur von Charité und MDC zur Unterstützung von Kooperationsprojekten und patientenbezogener Forschung. Es zielt darauf ab, vielfältige Verbindungen zwischen Grundlagenforschern und Klinikern herzustellen, um auf diese Weise den Wissensaustausch zwischen den beiden „Welten" zu fördern.

Das ECRC eröffnet Wissenschaftlern von Charité und MDC weitreichende Möglichkeiten zur Durchführung translationaler Projekte:

- *Kliniker oder Grundlagenforscher können sich um die Förderung einer unabhängigen Projektgruppe bewerben, um ein Translationsprojekt im ECRC durchzuführen. Die Förderung wird auf der Basis einer kompetitiven Begutachtung für drei Jahre mit der Möglichkeit zur Verlängerung vergeben.*
- *Kliniker und Grundlagenforscher können in einem ebenfalls kompetitiven Verfahren gemeinsam die Finanzierung kooperativer Projekte beantragen (Klinisches Kooperationsprogramm, KKP). Entsprechende Projekte werden in Räumen des ECRC und/ oder des MDC durchgeführt.*
- *MDC und Charité finanzieren im Rahmen des ECRC ein gemeinsames Ausbildungsprogramm (Klinisches Ausbildungsprogramm, KAP) für Kliniker in der molekularen Grundlagenforschung. So können Kliniker für einige Zeit ein Projekt in MDC-Laboratorien bearbeiten. Dadurch wird die Basis für eine spätere längerfristige Zusammenarbeit geschaffen.*
- *Kliniker betreiben Hochschulambulanzen am ECRC und führen klinische Studien durch.*
- *Klinische und experimentelle Forscher haben Zugang zu einem Klinischen Forschungszentrum*

Photo: David Ausserhofer / Copyright: MDC

Prof. Dr. Walter Rosenthal (MDC Scientific Director), Dr: Annette Schavan (German Minister of Education and Research), State Secretary Dr. Hans-Gerhard Husung (Berlin Senate for Education, Science, and Research), and Prof. Dr. Friedrich Luft (ECRC Director) (from left to right) at the dedication of the 7 Tesla ultra-high field magnetic resonance imaging (MRI) scanner and spectroscope facility on January 20, 2009 in a new research building of the MDC.

Prof. Dr. Walter Rosenthal (Wissenschaftlicher MDC-Vorstand), Bundesforschungsministerin Dr. Annette Schavan, Staatssekretär Dr. Hans-Gerhard Husung (Berliner Senatsverwaltung für Bildung, Wissenschaft und Forschung) und Prof. Dr. Friedrich Luft (ECRC-Direktor) (v. l.) bei der Einweihung des 7-Tesla-Magnetresonanztomographen am 20. Januar 2009 in einem neuen Forschungsgebäude des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch.

Scientific projects

Typically, ECRC projects have arisen in two ways. Some began as studies of molecular, cellular, or animal model systems related to disease, often at a very basic mechanistic or structural level. The second type of project began as work in which physicians were serving as caregivers for patients, who served as the starting point to probe mechanisms underlying particular health problems. A good example of the latter is the discovery by the group of Ludwig Thierfelder (MD) of a connection between mutations in the plakophilin gene and a condition called arrhythmogenic right ventricular cardiomyopathy. This gene was identified in the basic research lab of Walter Birchmeier (PhD) at the MDC, during a study of proteins involved in WNT signaling. Working together, the labs identified a mutation that greatly increases the likelihood of sudden cardiac arrest in affected people. This was developed into a diagnostic tool to identify family members at risk, who could be saved by the implantation of a pacemaker. These life-saving insights would not have been obtained without the constellation of a clinician interested in underlying disease mechanisms,

(Hochschulambulanzen, Probandenstation mit Betten für klinische Studien, siehe unten) und zu technolgischer Infrastruktur (bspw. Ultrahochfeld-Magnetresonanztomographie, GMP-Labor, Biobank)

Wissenschaftliche Projekte

Typischerweise sind ECRC-Projekte auf zweierlei Wegen entstanden: Ein Teil der Projekte hat mit oftmals sehr elementaren, mechanistischen oder strukturellen Untersuchungen auf molekularer bzw. zellbiologischer Ebene oder an Modellorganismen begonnen. Ein anderer Teil der Projekte resultiert aus der Betreuung von Patienten. Für den zweiten Weg ist die Entdeckung einer Verbindung zwischen Mutationen im Plakophilin-Gen und der arrythmogenen rechtsventrikulären Kardiomyopathie durch die Gruppe von Ludwig Thierfelder (Kliniker) ein gutes Beispiel. Das Gen wurde in der Forschergruppe von Walter Birchmeier (Zellbiologe) am MDC im Zusammenhang mit Arbeiten an Proteinen des sog. Wnt-Signalweges der embryonalen Entwicklung entdeckt. Im Rahmen einer Zusammenarbeit wurde eine Mutation bei Patienten arrythmogenen rechtsventrikulären Kardiomyopathie gefunden, die das Risiko eines plötzlichen Herzstillstandes stark erhöhte. Auf der Basis dieser Entdeckung wurde ein diagnostischer Test entwickelt, um innerhalb betroffener Familien Träger der Mutation zu identifizieren. Diese Personen können jetzt durch die Implantation eines Herzschrittmachers geschützt werden. Diese lebensrettenden Entdeckungen wären nicht gemacht worden, wenn es nicht das Zusammenspiel zwischen einem an den Krankheitsmechanismen interessierten Kliniker mit seinen Patienten und einem Zellbiologen gegeben hätte. Das Beispiel zeigt in eindrucksvoller Weise, wie das Konzept des ECRC zu einer Überwindung der traditionellen Trennung zwischen Grundlagenforschung und klinischer Medizin beitragen kann.

Alle klinischen Abteilungen der Charité und Forschungsgruppen des MDC haben die Möglichkeit, an ECRC-Projekten teilzunehmen. Allerdings erhalten sie Forschungsflächen im ECRC erst nach erfolgreicher Beantragung und Evaluierung durch Fachgutachter. Mehrere klinische Abteilungen forschen unter dem Dach des ECRC: Hämatologie und Onkologie (Bernd

his patients, and a basic research group. The case described is a prime example how the ECRC can overcome today´s principal problems deriving from the classical separation of basic science and clinical applications.

All clinical departments at the Charité and basic researchers of the MDC are eligible to participate in ECRC activities, but laboratory and clinical space is available only after proposals have been submitted to peer review and approved. Several major clinical groups are carrying out their work under the ECRC framework: the Department of Hematology-oncology (Bernd Dörken), the Department of Oncological surgery and the Charité Comprehensive Cancer Center (Peter Schlag), the Department of Neurology (Frauke Zipp, Simone Spuler), the Department of Cardiology (Rainer Dietz, Ludwig Thierfelder) and the Department of Nephrology-hypertension (Friedrich C. Luft). The ECRC also serves as the base for DFG-sponsored Clinical Research Groups in skeletal muscle diseases (directed by Simone Spuler) and malignant lymphoma (supported up to 2009; directed by Clemens Schmitt).

Some examples of clinical work currently underway within the ECRC are projects involving new types of therapies. For example, the clinicians Jörg Westermann and Antonio Pezzutto at the Charité, along with the laboratories of MDC/Charité researchers Bernd Dörken and Thomas Blankenstein, are carrying out phase 1 and phase 2 trials of immune therapies involving chronic myeloid leukemia and renal carcinomas.

Main components of the ECRC

The main physical components of the ECRC are a **clinical research center** (CRC) for studies involving patients and healthy humans, and a **high-field magnetic resonance facility** for the examination of **humans and model** animals. A third building to house the **experimental research center** (ERC), whose focus is disease-oriented experimental research, is planned.

The CRC, which is located on the Campus Buch in the Charité research building (the former Robert-Rössle-Clinic). Two outpatient clinics have already been established there: one for neuroimmunological dis-

Dörken), Onkologische Chirurgie und Charité Comprehensive Cancer Center (Peter Schlag), Neurologie (Frauke Zipp, Simone Spuler), Kardiologie (Rainer Dietz, Ludwig Thierfelder), Nephrologie und Bluthochdruck (Friedrich C. Luft). Das ECRC ist auch Basis der DFG-geförderten klinischen Forschergruppen auf dem Gebiet von Erkrankungen der Skelettmuskulatur (Leiterin: Simone Spuler) und des malignen Lymphoms (gefördert bis 2009; Leiter: Clemens Schmitt).

Einige laufende Projekte betreffen die Entwicklung neuer Therapiekonzepte. Zum Beispiel führen die Kliniker Jörg Westermann und Antonio Pezzuto zusammen mit MDC-Forschungsgruppen von Bernd Dörken und Thomas Blankenstein klinische Studien (Phase I und II) zur Immuntherapie chronischer myeloischer Leukämie und Nierenkarzinomen durch.

Komponenten des ECRC

Das ECRC verfügt über ein Gebäude für klinischen Forschung einschließlich Studien mit Patienten und Probanden (CRC) und ein Gebäude für Ultrahochfeld-Magnetresonanz-Bildgebung für Untersuchungen am Menschen und an Modellorganismen. Ein drittes Gebäude für die krankheitsorientierte, experimentelle Forschung (ERC) ist geplant.

Das CRC ist im Charité-Forschungsgebäude (ehemals Robert-Rössle-Klinik) auf dem Campus Buch untergebracht. Im CRC werden mehrere Hochschulambulanzen betrieben, eine für Neuroimmunologische Erkrankungen (mit Schwerpunkt Multiple Sklerose) und die andere für Muskelkrankheiten. Letztere wurde im Rahmen der DFG-geförderten Forschergruppe eingerichtet und behandelt im Jahr etwa 1200 Patienten mit seltenen Muskelerkrankungen. Weitere Hochschulambulanzen werden 2010 eingerichtet werden. Ergänzend können die Forscher des ECRC an allen Standorten der Charité Forschungsprojekte betreiben, die eine stationäre Aufnahme von Patienten erfordern.

Das CRC verfügt über Untersuchungsräume und spezielle Untersuchungsmethoden wie Mikroneurographie, Mikrodialyse, eine Kammer für Stoffwechseluntersuchungen, eine Hypoxie-Kammer sowie Vorrichtungen für invasives hämodynamisches Monitoring. Die zur Verfügung stehenden bildgebenden Verfahren

eases (with a special focus on multiple sclerosis) and another for muscle disease. The latter was established in the context of a DFG-financed clinical research group and deals with ca. 1200 patients with rare muscle diseases per year. Further outpatient clinics of the Charité will be established in 2010. In addition, ECRC researchers may conduct inpatient studies on all other Charité campuses.

The CRC has examining rooms, specialized procedure facilities such as microneurography, microdialyis, a metabolic chamber, normobaric hypoxia facilities, and tools for invasive hemodynamic monitoring. Available imaging technologies include ultrasound, echocardiography, Doppler tools, and access to high-field MRI. Personnel include study nurses, dietitians, biostatisticians, and ancillaries. The unit also has an erstwhile bone marrow transplant unit with appropriate safeguards. A "good manufacturing practice" (GMP) laboratory has been established to provide cellular therapies. At present, preparations are underway to launch a Phase I study for tumor immune therapy using dendritic cells, financed by the Helmholtz Association.

CRC physicians are trained in internal medicine, neurology, clinical pharmacology, and oftentimes a combination of these specialties. Credit towards certification in internal medicine, neurology, and clinical pharmacology can be granted to clinical trainees participating in the ECRC.

The CRC encompasses not only clinical research group projects but will include the Helmholtz Epidemiological Cohort study, for which 40,000 human subjects will be recruited from the Berlin region, from a total of 200,000 who are being recruited throughout Germany. Additionally, the CRC has the capacity to develop several outpatient clinics. Two outpatient clinics have already been established there: one for neuroimmunological diseases (with a special focus on multiple sclerosis) and another for muscle disease. The latter was established in the context of a DFG-financed clinical research group and deals with ca. 1200 patients with rare muscle diseases per year. Further outpatient clinics of the Charité will be established in 2010. In addition, ECRC researchers may conduct inpatient studies on all other Charité campuses.

umfassen Ultraschall, Echokardiographie, Doppler-Sonographie, und Hochfeld-Magnetresonanztomographie. Zum Personal gehören u. a. Studienschwestern, Diätassistenten und Biostatistiker. Ein GMP („good manufacturing practice") -Labor für die Zelltherapie wurde eingerichtet. Gegenwärtig wird eine Phase I-Studie zur Immuntherapie mit dendritischen Zellen vorbereitet, die von der Helmholtz-Gemeinschaft finanziert wird.

Die ärztlichen Mitarbeiter des CRC erhalten eine Ausbildung in Innerer Medizin, Neurologie oder Klinischer Pharmakologie, meist als Kombination dieser Fächer, die sie sich auf ihre Facharztausbildung anrechnen lassen können.

Im CRC werden nicht nur die Projekte klinischer Forschungsgruppen durchgeführt sondern es wird auch die epidemiologische Kohortenstudie der Helmholtz-Gemeinschaft beherbergen, für die aus der Berliner Region 40000 Probanden rekrutiert werden sollen. Insgesamt sollen deutschlandweit 200000 Probanden in die Kohorte eingeschlossen werden.

Die Ultrahochfeld-Magnetresonanz-Anlage des MDC mit einem 7 Tesla-Ganzkörper-Magnetresonanztomograph (MRT) für die Anwendung am Menschen und einem experimentellen 9,4 Tesla-MRT ist im Januar 2009 eröffnet worden. Bis Ende 2009 wurde das Gebäude um ein zweites Stockwerk für die Unterbringung eines klinischen 3 Tesla-MRT erweitert. Damit stehen nunmehr insgesamt 913 m Forschungsfläche für die MR-Bildgebung zur Verfügung. Der Physiker, Thoralf Niendorf, ein führender Experte auf dem Gebiet der MR-Technologie mit Erfahrung im akademischen und industriellen Bereich wurde als Leiter der Ultrahochfeld-MR-Anlage berufen.

In unmittelbarer Nachbarschaft der Ultrahochdfeld-MR-Anlage wird das MDC im Frühjahr 2010 mit dem Neubau für das ERC beginnen. Nach Fertigstellung werden im ERC weitere ca. 2600 m^2 hochwertige und flexibel nutzbare Labor- und Büroflächen für die translationale Forschung zur Verfügung stehen.

Ausbildungsziele

Ein wichtiges Motiv für die Gründung des ECRC war die Notwendigkeit, eine neue Generation von For-

The ultrahigh field MR facility was opened in January 2009. By the end of 2009, the facility has been enlarged by a second floor to house a new 3 Tesla clinical scanner in addition to a 9.4 Tesla animal scanner and a 7 Tesla whole body scanner, resulting in a total of 913 m of research space for MR technologies. Thoralf Niendorf was appointed director of the facility in 2009. A physicist by training, Thoralf Niendorf is a leading expert in MR imaging with a research experience in academia and industry.

To house the ERC, the MDC will start construction on a new building in close proximity to the MR facility in spring 2010. The ERC building will comprise ca. 2600 m^2 of highly flexible laboratory and office space for translational research projects.

Educational mission

An important motivation for the project is the need for a new breed of researcher: a "physician-scientist" equally at home in the clinic and the basic research lab. Translational research involves all levels of biological organization, from the structures and functions of single molecules to the overall health of an organism over the long term, Physicians and basic researchers have complementary perspectives and expertise that need to be combined – ideally in a single person – to design meaningful basic experiments that will shed light on disease processes in patients.

The centerpiece of the educational activities is a training program for clinicians in basic molecular biology, jointly sponsored by the MDC and Charité, in which clinicians take "time out" from their clinical duties and join a laboratory run by an MDC basic scientist. Entry into the program (*Kliniker Ausbildungsprogramm*, or KAP, in German) is gained through a competitive application procedure. Proposals are peer-reviewed with intermittent follow-up. Successful applications commonly lead to long-term collaborations between a clinical group and the MDC basic science laboratory hosting the applicant.

Perspectives

The ECRC will provide vital infrastructure for some other projects that are currently being planned, such as the establishment of a German Center for Cardiovascular Research (Deutsches Zentrum für

schern auszubilden, die gleichermaßen in der Klinik wie im Forschungslabor zu Hause sind. Die translationale Forschung spannt den Bogen von der Struktur und Funktion einzelner Moleküle bis zur ganzheitlichen Funktion des Organismus. Ärzte und experimentelle Biologen haben einander ergänzende Denkweisen und Erfahrungen, die vereinigt werden müssen – idealerweise in einer Person – wenn Experimente so konzipiert sein sollen, dass sie zu neuen Einsichten über Krankheitsprozesse in Patienten führen können.

Eine Schlüsselkomponente des ECRC ist daher ein Trainingsprogramm, um den „Physician Scientist" mit dem entsprechenden Expertise-Profil auszubilden. Ein zentrales Element ist hierbei das Ausbildungsprogramm für Kliniker in der Molekularbiologie, das von Charité und MDC gemeinsam gestaltet wird. Hier können Kliniker eine „Auszeit" von ihren klinischen Pflichten nehmen und in einem Labor arbeiten, das von einem MDC-Forscher geleitet wird. Der Zugang zum Kliniken Ausbildungsprogramm (KAP) ist über ein kompetitives Bewerbungsverfahren geregelt. Der Projektfortschritt wird durch eine Zwischenevaluation überprüft. Gleichzeitig bildet der Gastaufenthalt oftmals die Ausgangsbasis für eine längerfristige Zusammenarbeit zwischen dem KAP-Stipendiaten und der gastgebenden Abteilung.

Perspektiven

Das ECRC wird wichtige Infrastrukturkomponenten für weitere Projekte bereitstellen, die sich gegenwärtig im Planungsstadium befinden, bspw. ein Deutsches Zentrum für Kardiovaskuläre Forschung (DZHK) und ein Deutsches Institut für Kardiovaskuläre Forschung (DIHK, wie in der Einleitung zum Research Report erwähnt). Wenn das DIHK wie vorgesehen auf dem Campus Buch angesiedelt wird, könnte es von der unmittelbaren Nachbarschaft des ECRC mit seinen technologischen Plattformen und seiner Forschungsinfrastruktur profitieren.

Kardiovaskuläre Forschung, DZHK) and a German Institute for Cardiovascular Research (Deutsches Institut für Kardiovaskuläre Forschung, DIHK) which have been proposed by the MDC and Charité. The proposal foresees the construction of the DIHK on the Berlin-Buch campus, which would put it in the immediate vicinity of the ECRC and its technological platforms and infrastructure.

Structure of the ECRC

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Thomas Sommer

Selected publications of ECRC participants

Mathas, S, Kreher, S, Meaburn, KJ, Jöhrens, K, Lamprecht, B, Assaf, C, Sterry, W, Kadin, ME, Daibata, M, Joos, S, Hummel, M, Stein, H, Janz, M, Anagnostopoulos, I, Schrock, E, Misteli, T, Dörken, B. (2009) Gene deregulation and spatial genome reorganization near breakpoints prior to formation of translocations in anaplastic large cell lymphoma. Proc Natl Acad Sci U S A. 106, 5831-6.

Stein, U, Walther, W, Arlt, F, Schwabe, H, Smith, J, Fichtner, I, Birchmeier, W, Schlag, PM. (2009) MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. Nat Med. 15, 59-67.

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Machnik, A, Neuhofer, W, Jantsch, J, Dahlmann, A, Tammela, T, Machura, K, Park, JK, Beck, FX, Müller, DN, Derer, W, Goss, J, Ziomber, A, Dietsch, P, Wagner, H, van Rooijen, N, Kurtz, A, Hilgers, KF, Alitalo, K, Eckardt, KU, Luft, FC, Kerjaschki, D, Titze, J. (2009) Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. Nat Med. 15, 545-52.

Organisationsstruktur des ECRC

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Ausgewählte Publikationen von Forschern des ECRC

Mathas, S, Kreher, S, Meaburn, KJ, Jöhrens, K, Lamprecht, B, Assaf, C, Sterry, W, Kadin, ME, Daibata, M, Joos, S, Hummel, M, Stein, H, Janz, M, Anagnostopoulos, I, Schrock, E, Misteli, T, Dörken, B. (2009) Gene deregulation and spatial genome reorganization near breakpoints prior to formation of translocations in anaplastic large cell lymphoma. Proc Natl Acad Sci U S A. 106, 5831-6.

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Hauck, L, Harms, C, An, J, Rohne, J, Gertz, K, Dietz, R, Endres, M, von Harsdorf, R. (2008) Protein kinase CK2 links extracellular growth factor signaling with the control of p27(Kip1) stability in the heart. Nat Med. 14, 315-24.

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Simone Spuler

Structure of the Group

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PD Dr. Hans Knoblauch
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Dr. Stephanie Adams
Dr. Miriam Carl

Muscle Research Unit and Clinical Research Group (CRG) 192

Simone Spuler directs CRG 192 for skeletal muscle diseases (speaker, Friedrich C. Luft) at the Charité. CRG 192 is home to groups at all Charité campuses; however, much of the research activities, including her own research team is located at the ECRC. Within the ECRC, she directs an outpatient clinical for >1000 patients with genetic and acquired skeletal muscle diseases. CRG 192 is in the process of expanding by founding a graduate school (Graduiertenkolleg) for doctoral and postdoctoral students in conjunction with the University of Paris. Simone Spuler has focused her attention on dysferlin. The dysferlinopathies encompass a large variety of neuromuscular diseases characterized by the absence of dysferlin in skeletal muscle and an autosomal recessive mode of inheritance. Three main phenotypes are known, Miyoshi myopathy, limb girdle muscular dystrophy type 2B, and distal myopathy with anterior tibial onset.

Dysferlin is a large protein located at the sarcolemma and is involved in membrane repair after microinjury, a physiological consequence of normal muscle activity. Patients with mutations in the dysferlin gene experience progressive muscle weakness in early adulthood leading to loss of ambulation within 10-15 years. Soon after the dysferlin gene was discovered in 1998, inflammatory changes were observed to be an intrinsic part of the disease spectrum in dysferlinopathies. The Spuler laboratory was the first to demonstrate involvement of the immune system. They observed that the complement inhibitory factor CD 55 is selectively downregulated on dysferlin-deficient skeletal muscle leading to increased vulnerability to complement attacks. Modulation of the complement cascade may open a therapeutic strategy.

The team was also the first to demonstrate that dysferlin mutations lead to misfolding and aggregation of dysferlin and muscle amyloidosis. In many aspects, dysferlinopathies resemble other protein misfolding diseases such as Alzheimer's disease and Parkinson's disease. Dr. Verena Schöwel is working on a strategy to rescue missense-mutated dysferlin from degradation and relocating the molecule to the plasma membrane. Dr. Ute Zacharias is investigating the effect of the potent modulator of skeletal muscle growth, myostatin, on dysferlin-deficient human myotubes. These efforts are leading to novel treatments to relocate the available protein. An effort is underway to apply this new knowledge to in vivo models.

Muscular dystrophies are closely related to generalized disturbances in metabolism. Years ago, muscular dystrophies were considered to be endocrinological diseases. This aspect was later neglected due to the enormous progress in visualization techniques, genetic tools, and molecular biology. The structural abnormalities of skeletal muscle disorders were described and categorized. Furthermore, many new genes were identified that cause myopathies once mutated. However, the

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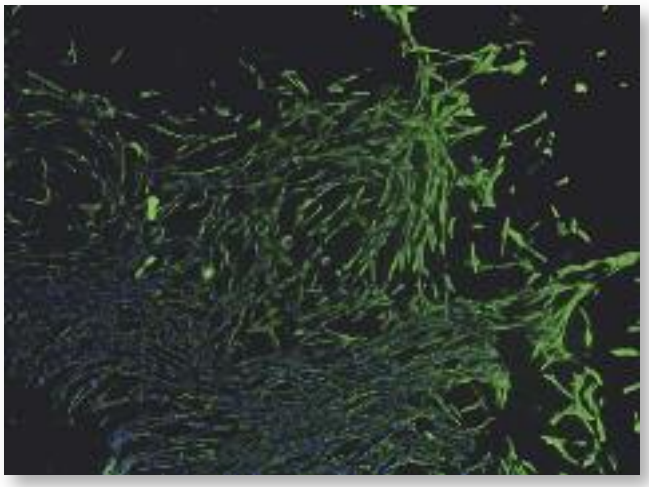

Hundreds of myoblasts can be generated from a single human muscle fiber. Mouse anti-human desmin ab (green) and Hoechst stain.

mechanism of progressive muscle weakness and replacement of muscle by connective tissue is still not well understood. Today, we have increasing evidence that important aspects of muscular dystrophies may indeed be endocrinological. In an interdisciplinary approach involving pharmacologists, biochemists, endocrinologists and neurologists we investigate glucose and lipid metabolism in vivo and in vitro in molecularly defined groups of patients. Some muscular dystrophies, Dunnigan's for example, feature partial lipodystrophy, early onset of type 2 diabetes mellitus, and non-alcoholic steatosis. The Spuler group is working together with other ECRC investigators belonging to CRG 192 to elucidate the faulty metabolism of these disorders. Joanna Schneider is working on FRET analysis using glucose sensitive vectors.

After muscle injury, muscle satellite cells have the full potential to proliferate, differentiate, and conduct repair. However, in tissue culture these cells rapidly lose these properties. Because satellite cells would be a good target for gene therapy, it is important to investigate their regenerative potential in vitro. Much information is available from mouse models. Human satellite cells have not been characterized in any meaningful detail. Dr. Stephanie Adams and Sina Gloy isolate and characterize human satellite cells. They are focusing in particular on Notch and Wnt signaling pathways. The potential of this work is particularly relevant for the ECRC with the advent of the GMP stem cell laboratory, a core facility for all disciplines at the ECRC and MDC. When the time comes, the movement to translate new findings will be rapid.

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Ralph Kettritz

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Dr. Mira Choi
Dr. Julia Bontscho
Dr. Uwe Jerke
Dr. Astrid Bergmann
Claudia Eulenberg

Neutrophil biology in health and disease

This group focuses on the neutrophilic granulocyte. Neutrophils form an essential part of the innate immune system. Neutrophils circulate in the blood stream and migrate into tissues during inflammation and also in some cancers. This process is initiated by signals, such as Interleukin-8 (IL-8), Interferon-gamma (IFN-γ), and complement fraction C5a. The fundamental finding that some systemic vasculitidis are caused by autoantibodies directed at neutrophil proteinase 3 and myeloperoxidase, sparked our interest in neutrophils. These antineutrophil cytoplasmic antibodies (PR3 and MPO ANCA) cause a common syndrome featuring upper and lower airway disease as well as rapidly progressive "pauci-immune" glomerulonephritis (ANCA vasculitis). In addition, our group is interested in all aspects of neutrophil function.

A mouse model of ANCA vasculitis

Adrian Schreiber, a DFG-sponsored research fellow, participated in the development of a mouse model during his fellowship at the University of North Carolina with Charles Jennette. They immunized MPO gene-deficient mice with MPO inducing MPO ANCA. Then, these mice were irradiated and underwent bone-marrow transplant with MPO+/+ marrow. The animals now had MPO ANCA and MPO in their neutrophils and developed a classic MPO ANCA vasculitis, including glomerulonephritis. We recently made the novel observation that the C5a receptor mediates neutrophil activation in this model. Supernatants from ANCA-stimulated neutrophils activated the complement cascade in normal serum in vitro, producing C5a. This conditioned serum primed neutrophils for ANCA-induced activation and C5aR blockade abrogated this effect. In our mouse model, bone marrow transplants were done from C5a-/- and C5a+/+ mice. The results were convincing and showed that the complement system is pivotal to MPO ANCA vasculitis raising important therapeutic possibilities.

The CD177 glycoprotein NB1 and PR3 cell-surface presentation

The serine protease proteinase 3 (PR3) is a main autoantigen in ANCA vasculitis. PR3 surface presentation on neutrophilis, the main effector cells, is pathogenically important. PR3 is presented by the NB1 (CD177) glycoprotein, but how the presentation develops during neutrophil differentiation is not known. The Kettritz team is intensively studying the relationship between NB1 and PR3. An N-terminally unprocessed PR3 (proPR3) is produced early during neutrophil development and promotes myeloid cell differentiation. The group recently tested whether or not it depended on NB1 during neutrophil differentiation and if PR3 and proPR3 could both be presented by NB1. NB1-mediated PR3 presentation depended on PR3 N-terminal processing implicating the PR3-N-terminus as NB1-binding site. Kettritz and associates are currently pursuing this promising area of investigation further.

The adage is "starve a fever, feed a cold."

Physicians spend much of their time fighting fever; even William Osler thought that the issue was important. We asked whether or not fever regulates neutrophil behavior in a series of studies. Mira Choi was primarily responsible for this work. The group tested the hypothesis that exposure of mice to fever-like temperatures abrogates neutrophil recruitment and NF-kappaB activation in a mouse model of skin inflammation. Mice

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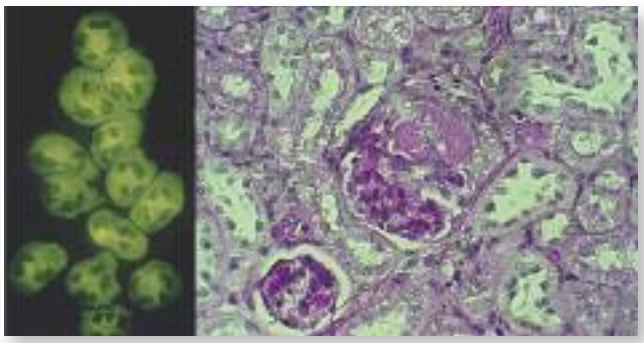
that were exposed to 40° C for 1 hour showed strongly reduced GM-CSF- and IL-8-induced neutrophilic skin inflammation. In vitro heat exposure abrogated migration toward both cytokines by down-regulating PI3-K/Akt. Furthermore, neutrophils on fibronectin showed abrogated NF-κB activation in response to GM-CSF and IL-8 after heat. Less NF-κB activation was seen in the inflammatory lesions of mice exposed to fever-like temperatures as demonstrated by in situ hybridization for IκBα mRNA. These new findings suggest that heat may have anti-inflammatory effects in neutrophil-dependent inflammation. Thus, we believe we should let fevers be!

Is platelet dust more important than stardust?

Birgit Salanova and the group tested the notion that platelets could "spill over" functional glycoprotein IIb/IIIa (GPIIb/IIIa) receptors onto neutrophils via platelet-derived microparticles (PMPs). The group observed that acquired GPIIb/IIIa receptors co-localized with beta2-integrins and cooperated in NF-κB activation. They showed that Src and Syk non-receptor tyrosine kinases, as well as the actin cytoskeleton, control NF-kappaB activation. When granulocyte macrophage colony-stimulating factor-treated neutrophils were incubated on fibronectin, strong NF-κB activation was observed, but only after loading with PMPs. Currently available GPIIb/IIIa inhibitors were effective. The data implicate GPIIb/IIIa receptors as new therapeutic targets in neutrophil-induced inflammation.

BK channels and neutrophil function

The group (in collaboration with Maik Gollasch (ECRC) and William Nauseef (University of Iowa) was involved in an "intensive" controversy with a group at University College London, regarding the importance of BK channels in neutrophil "burst" reactions. To test this notion, Kirill Essin and the group directly assessed the role of BK channels in neutrophil function, including the NADPH oxidase. Neutrophils lacking BK channels (BK-/-) had normal NADPH oxidase activity in response to receptor-independent and phagocytic challenges. Furthermore, NADPH oxidase activity of neutrophils and macrophages was normal after treatment with BK channel inhibitors. The group concluded that the BK channel is not required for NADPH oxidase activity in neutrophils. The contentious argument has been dropped.



ANCA from a patient with active vasculitis shows a cytoplasmic immunofluorescence staining pattern in permeabilized neutrophils (left). Necrotizing crescentic glomerulonephritis in a mouse model of ANCA vasculitis (right).

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Jeanette Schulz-Menger

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Dr. Florian v. Knobelsdorff-Brenkenhoff

Cardiovascular Magnetic Resonance

The CMR group at the Franz-Volhard-Klinik has focused its research on the in vivo assessment of functional and structural myocardial abnormalities related to inflammatory diseases and coronary heart disease. We perform clinical research using a 1.5 clinical MRI-scanner with dedicated cardiac software. We developed new approaches for the differentiation of tissue changes in myocardial diseases. The application as research tools and the translation into a clinical setting are the main interests of the group. The successful work led to the introduction of the University Professorship in Cardiology for Noninvasive Imaging focused on Cardiovascular magnetic resonance (CMR).

In 2009 the Ultra-High-Field Facility was opened giving us the possibility to extend our research field by developing translational research tools applying advanced imaging modalities.

Myocardial Injury

The diagnosis of myocardial inflammation in different diseases and the assessment of myocardial tissue changes during follow-up is a challenging task in cardiovascular research and clinical cardiology. Clinical presentation of patients with myocarditis or myocardial involvement in systemic disorders often mimics other disorders and may vary from flu-like symptoms or subclinical disease to acute heart failure and sudden cardiac death. Cardiovascular magnetic resonance (CMR) has the capability to differentiate between the various forms of myocardial injuries (e.g. edema, hyperemia and fibrosis). In the late 90s we developed an approach for the noninvasive detection of acute myocarditis by CMR. In 2008/09 we published the noninvasive detection of myocardial edema and the capability of a multi-sequential approach for follow-up.(1) Myocardial inflammation also has a high prognostic impact in different systemic diseases. However early assessment is difficult. We used CMR technology in patients with systemic lupus erythematosus and Churg Strauss syndrome and were able to detect myocardial involvement in those patients with preserved left-ventricular function. (2) It is well known, that that development of fibrosis is of potential prognostic impact in hypertrophy. Using contrast enhanced CMR we were able do differentiate the pattern in various non-ischemic disease (3) and visualize fibrotic tissue in hypertrophy caused by different diseases (4) Furthermore we tried to get insight the gender-related remodeling process in hypertrophic cardiomyopathy. (5)

Coronary Artery Disease and Arteriosclerosis

The detection of coronary artery stenosis is a growing field in CMR. Quantitative analysis of perfusion is a crucial step for evaluation of significant ischemia and development of new therapeutic strategies. In 2009 we could show that the lipid-apheresis has an impact on myocardial perfusion in young patients with elevated LPa. In 2009 we published, that the quantitative dual bolus perfusion is of higher accuracy having the potential to reduce the sample size. The field is our step into Ultra-High-Field research. In collaboration

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Dr. Ralf Wassmuth
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Evelyn Polzin

Study nurse/Project assistance
Annette Köhler

with the University of Oxford we published our first paper on CMR-driven infarct-detection in mices. (6) We are working on establishing the method at the MDC, Technical improvement will take place at all levels of research in a strong collaboration with the group of the Ultra-Highfield-Facility

Selected publications

Zagrosek A, Abdel-Aty H, Boyé P, Wassmuth, R. Messroghli, DR. Utz, W. Rudolph, A. Bohl, S. Dietz, R. Schulz-Menger, J. Cardiac Magnetic Resonance Monitors Reversible and Irreversible Myocardial Injury in Myocarditis. J Am Coll Cardiol CVI 2009;2(2):131-138

Bohl S, Wassmuth R, Abdel-Aty H, Rudolph, A. Messroghli, D. Dietz, R. Schulz-Menger, J.Delayed enhancement cardiac magnetic resonance imaging reveals typical patterns of myocardial injury in patients with various forms of non-ischemic heart disease. Int J Cardiovasc Imaging. Aug 2008;24(6):597-607.

Rudolph A, Abdel-Aty H, Bohl S, Boye, P. Zagrosek, A. Dietz, R. Schulz-Menger, J. Noninvasive detection of fibrosis applying contrast-enhanced cardiac magnetic resonance in different forms of left ventricular hypertrophy relation to remodeling. J Am Coll Cardiol. Jan 20 2009;53(3):284-291.

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Maik Gollasch

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Dr. Galyna Dubrovska

Technical Assistants
Yoland-Marie Anistan
Diana Herold

Manager of sponsored programs
Susanne Wissler

Smooth Muscle Cell Electrophysiology, Ion Channel, and Transporter Function

Ion channels and electrophysiology

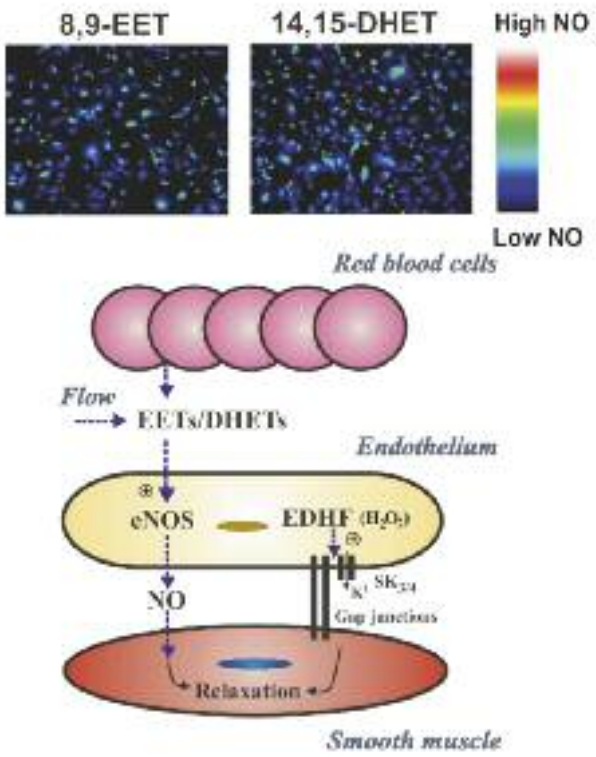
This group focuses on ion channels, primarily in vascular smooth muscle cells (VSMC), to clarify mechanisms contributing to hypertension and cardiovascular disease. Calcium-activated potassium (BK) channels have received special attention. The group collaborated with Ralph Kettritz to study the notion that BK channels are involved in neutrophil activation. A knockout mouse model showed convincingly that BK channels are not involved in this process. The group investigated the role of transient receptor potential (TRP) ion channels in agonist-independent G(q 11) protein-coupled receptor activation. Their work showed that G(q 11)-coupled receptors could function as membrane stretch receptors in VSMC. A close collaboration with Wolf-Hagen Schunck at the MDC has turned the group's focus towards eicosanoids. The group found that p450 eicosanoids such as epoxyeicosatrienoic acids are vasodilatory, largely through their ability to activate endothelial NO synthase and NO release. Furthermore, in collaboration with Huang Yu in Hong Kong, they found that endothelium-derived contracting factors are dependent on cyclooxygenase-2. Another area of research is directed towards identifying the role of the perivascular fat as a modulator of arterial tone, with specific emphasis on the resistance vasculature.

BK channels and neutrophil function

The group (in collaboration with Ralph Kettritz (ECRC) and William Nauseef (University of Iowa) was involved in an "intensive" controversy with a group at University College London, regarding the importance of BK channels in the mediation of neutrophil "burst" reactions. To test this notion, Kirill Essin and the group directly assessed the role of BK channels in neutrophil function, including the NADPH oxidase. Neutrophils lacking BK channels (BK-/-) had normal intracellular and extracellular NADPH oxidase activity in response to both receptor-independent and phagocytic challenges. Furthermore, NADPH oxidase activity of neutrophils and macrophages was normal after treatment with BK channel inhibitors. Although BK channel inhibitors suppressed endotoxin-mediated tumor necrosis factor-alpha secretion by bone marrow-derived macrophages, cells from BK-/- and wild-type mice responded identically and exhibited the same ERK, PI3K/Akt, and NF-κB activation. The group concluded that the BK channel is not required for NADPH oxidase activity in neutrophils. The contentious argument has been dropped.

Epoxyeicosatrienoic acids (EETs)

EETs serve as endothelial-derived hyperpolarizing factors (EDHF), but may also affect vascular function by other mechanisms. The Gollasch team identified a novel interaction between EETs and endothelial NO release using soluble epoxide hydrolase (sEH) -/- and +/+ mice. EDHF responses to acetylcholine in pressurized isolated mesenteric arteries were neither affected by the sEH inhibitor, N-adamantyl-N'-dodecylurea (ADU), nor by sEH gene deletion. However, the EDHF responses were abolished by catalase and by apamin/charybdotoxin (ChTx), but not by iberiotoxin, nor by the cytochrome P450 inhibitor PPOH. All four EETs (order of potency: 8,9-EET >14,15-EET approximately 5,6-EET >11,12-EET) and all 4 dihydroxy derivatives (14,15-DHET approximately 8,9-DHET approximately 11,12-DHET >5,6-DHET) produced dose-dependent vasodilation. Endothelial removal or L-NAME blocked 8,9-EET and 14,15-DHET-dependent dilations. The effects of apamin/ChTx were minimal. 8,9-EET and 14,15-DHET induced NO production in endothelial cells. ADU (100 µg/ml in drinking water) lowered blood pres-



Epoxyeicosatrienoic acids (EETs) and dihydroxyeicosatrienoic acids (DHETs) stimulate the production of nitric oxide (NO) in endothelial cells to induce vasorelaxation. Red blood cells are a rich source of EETs and may utilize this mechanism to produce vasorelaxation in the microcirculation (from the cover page of Arterioscler Thromb Vasc Biol. 2009 Jan;29(1)).

sure in angiotensin II-infused hypertension, but not in L-NAME-induced hypertension. Blood pressure and EDHF responses were similar in L-NAME-treated sEH +/+ and -/- mice. The data indicate that the EDHF response in mice is caused by hydrogen peroxide, but not by P450 eicosanoids. Moreover, P450 eicosanoids are vasodilatory, largely through their ability to activate endothelial NO synthase (eNOS) and NO release.

Endothelial-derived contracting factors (EDCFs)

Hypertension and vascular dysfunction result in the increased release of EDCFs, whose identity is poorly defined. The Gollasch team tested the hypothesis that endothelial cyclooxygenase (COX)-2 can generate EDCFs and identified the possible EDCF candidate. They showed that endothelium-dependent contractions were triggered by acetylcholine (ACh) after inhibition of nitric oxide production and they were abolished by COX-2 but not COX-1 inhibitors or by thromboxane-prostanoid receptor antagonists. The cation channel blocker, 2-amino-ethoxydiphenyl borate eliminated endothelium-dependent contractions and ACh-stimulated rises in endothelial cell [Ca(2+)](i). RT-PCR and Western blotting showed COX-2 expression mainly in the endothelium. Enzyme immunoassay and high-performance liquid chromatography-coupled mass spectrometry showed release of prostaglandin (PG)F(2alpha) and prostacyclin (PGI(2)) increased by ACh; only PGF(2alpha) caused contraction at relevant concentrations. COX-2 expression, ACh-stimulated contractions, and vascular sensitivity to PGF(2alpha) were augmented in aortae from aged hamsters. Human renal arteries also showed thromboxane-prostanoid receptor-mediated ACh- or PGF(2alpha)-induced contractions and COX-2-dependent release of PGF(2alpha). The results support a critical role of COX-2 in endothelium-dependent contractions in this species with an increased importance during aging and, possibly, a similar relevance in humans.

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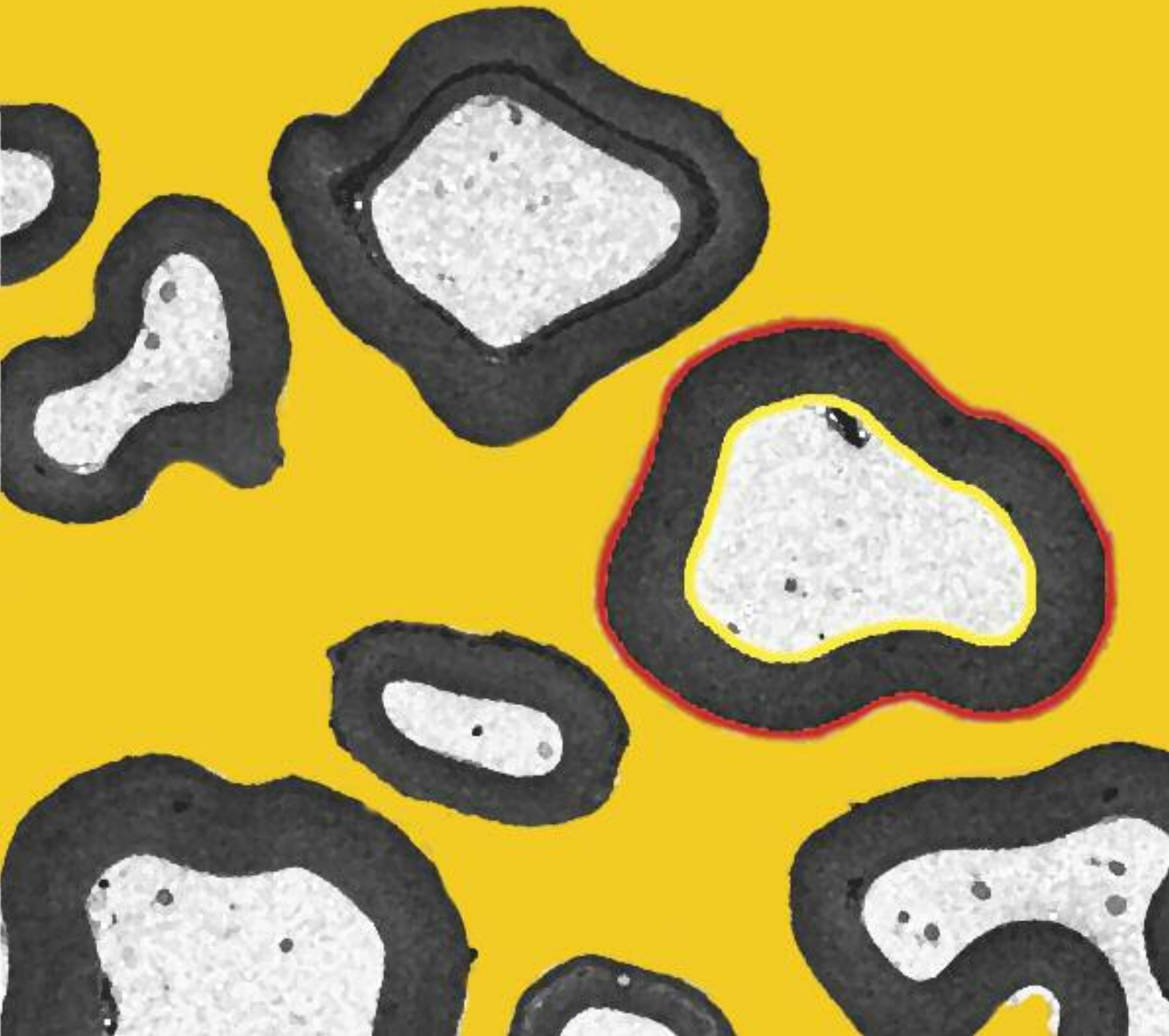
Technology Platforms

Computational Biology and Data Mining
Mathematical Cell Physiology
Mass Spectrometry
Preparative Flowcytometry
Electron Microscopy
Transgenics



Miguel Andrade

Structure of the Group

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Dr. Adriano Barbosa
Dr. Nancy Mah

Graduate Student
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Technical Assistant
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Secretariat
Sylvia Olbrich
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Computational Biology and Data Mining

Our group focuses on the development and application of computational methods that are used to research the molecular and genetic components of human disease. Often, we work with data from high throughput gene expression, proteomics, and protein-protein interaction experiments performed at a genomic level. Our primary research tools, besides hardware and software, are the increasing collection of public repositories of biological information such as molecular sequence and structure databases, literature databases like MEDLINE, and other resources related to human disease such as the OMIM database. The results of our work are distributed as software or online web services.

Prediction of transcript 3'UTR ends

Methodologies for the prediction of gene transcripts from genomic and expression data are still under development, and the increase in the amount of transcript data in public databases offers a chance to improve such methods. We observed that the databases of expressed sequence tags (ESTs) contain abundant evidence of alternative 3'UTR ends that are currently absent from the public database records for many genes, or invalidate many transcript ends included in databases like RefSeq or Ensembl. We proposed and experimentally verified a method to predict transcript ends using EST data and analysis of poly-adenylation signals (Muro et al., 2008). The results of the analysis of the complete human and murine genomes are available as data tables and through the Transcriptome Sailor web tool [http://www.ogic.ca/ts/], which allows examination of particular genomic regions for predictions and evidence.

Analysis of gene expression in stem cell differentiation

Gene expression repositories generated for a particular objective within a single laboratory overcome some of the problems that pervade such data such as platform heterogeneity and poor sample quality. The Stem Cell Genomics Project (http://www.ottawagenomecenter.ca/projects/stemcellgenomics) used this approach to produce a resource of gene expression experiments that follow stem cell differentiation. In this context, we produced a database of stem cell microarray data [StemBase; http://www.stembase.ca/] describing gene expression (from cDNA microarrays and experiments of serial analysis of gene expression) in more than 200 samples of stem cells and their derivatives in mouse and human (Sandie et al., 2009). We illustrated how to use these data to study stem cell biology at three levels: cells, genetic networks, and particular genes. The database provides a variety of querying mechanisms for different tasks including the detection of gene markers [http://www.ogic.ca/projects/markerserver/] and the use of the UCSC Genome Browser to display the data in genomic context.

Identification of protein repeats

Since 1995, when we found the first homology of the Huntington's disease protein (huntingtin) to other protein sequence to be a repeat of around 40 amino acids (HEAT repeat; Andrade and Bork, 1995), few advances have been made in the characterization of these repeats in huntingtin or in other proteins. In the case of huntingtin, this enormously hampers the elucidation of its normal function and of the mechanisms that trigger Huntington's chorea. To approach this problem, we developed a neural network [ARD; http://www.ogic.ca/

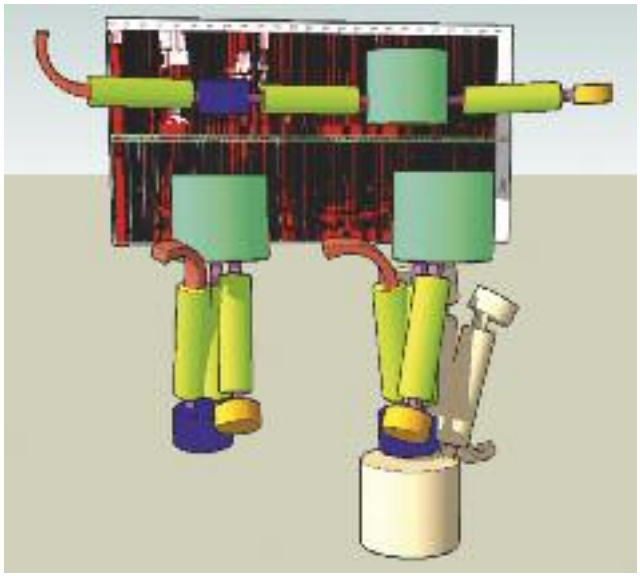
projects/ard/] to detect repeats like HEAT, Armadillo, and PBS, that form similar structures composed of alpha-helices (which we termed alpha-rods) (Palidwor et al., 2009). This method allowed us to detect novel instances of this structure, for example in human proteins STAG1-3, SERAC1, and PSMD1-2 & 5. Application of the method to human huntingtin and comparison to orthologs allowed us to delimit three alpha-rods in huntingtin (See Figure 1) whose intra-molecular interactions we characterized experimentally using yeast two hybrid and co-immunoprecipitation of protein fragments encoding the domains.

Protein domain analysis

Some of our work focuses on the search for particular protein functions using protein sequence comparison and other bioinformatics tools. A recent example is our search for proteins regulating mitochondrial morphology where we did a computational screen for protein sequences predicted as mitochondrial and containing RING domains (characteristic of ubiquitinating enzymes). We experimentally characterized one of them, which we named MAPL (mitochondrial anchored protein ligase), which is located in the outer membrane of the mitochondria. Observation of MAPL-YFP led to the first observation of mitochondrially derived vesicles, which fuse with peroxisomes. The protein contains a domain that we named BAM (Besides a Membrane) (Andrade-Navarro et al., 2009), which presents a complicated taxonomic distribution, being scattered in archaea and bacteria, and present in most eukarya (but not in fungi). We deduce that this domain has been generated along the eukaryotic lineage and has been horizontally transferred multiple times to and between prokaryotic lineages. We hypothesize that it must have a function that confers prokaryotes with a selective advantage without being crucial.

Data and text mining

Databases of molecular sequences are a very useful source of functional information for genes and proteins but nevertheless they are often incomplete or not up to date. This motivates us and many others working in the field of computational biology to develop tools that "mine" resources that contain textual descriptions of research results, chiefly, the millions of abstracts referring to the biomedical literature deposited in the MEDLINE database. In this respect, we recently developed a scoring system to rank all abstracts in MEDLINE according to a training set that can consist of ten of thousands of abstracts (Fontaine et al., 2009); this is useful



Using a computational method on a sequence alignment of huntingtin with its orthologs (output in the background) we predicted that the human huntingtin contains three elongated domains (alpha-rods, yellow cylinders), which are involved in intra-molecular (left) and inter-molecular (right) interactions, liberally represented in this cartoon.

when a researcher has a small dataset of references of interest and wants to find "more of the same". Algorithms such as neural networks or support vector machines are too computationally intensive for this task so we opted for a simpler solution that could be defined as text indexing. We implemented and evaluated this method using a linear naïve Bayesian classifier in a web server [MedlineRanker; http://cbdm.mdc-berlin.de/~medlineranker/]. This tool uses as input a set of MEDLINE abstracts and optionally a background to compare to, and outputs discriminate words and scored abstracts.

Selected Publications

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Sandie, R, Palidwor, GA, Huska, MR, Porter, CJ, Krzyzanowski, PM, Muro, EM, Perez-Iratxeta, C, Andrade-Navarro, MA. (2009). Recent developments in StemBase: a tool to study gene expression in human and murine stem cells. BMC Research Notes. 2, 39.

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Martin Falcke

Structure of the Group

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Start of group: March 2009

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Diploma Student
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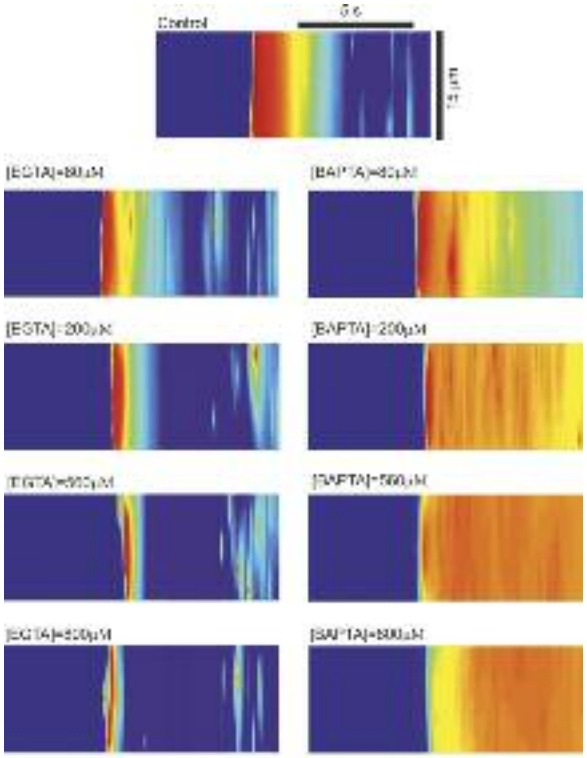
Mathematical Cell Physiology

The core facility group „Mathematical Cell Physiology" develops mathematical models of cellular processes. Current projects comprise second messenger signaling systems (cAMP, Ca^{2+}), membrane potential dynamics, pH-regulation, cardiac myocyte cell models, actin dynamics and cell motility. The group's service to experimental groups is to develop quantitative formulations of their hypotheses in form of mathematical models. We provide that service on a variety of levels from assistance in usage of modeling tools to complete model formulation and testing. The group works currently with the labs of T. Jentsch's, I. Morano's and H. Kettenmann's. We also conduct independent research. Projects on IP_3-induced Ca^{2+}-signaling, cAMP-signaling and actin dynamics were the focus in 2008/2009. All studies reported here were close collaborations with experimental groups.

In many cell types, the inositol trisphosphate receptor IP_3R is one of the important components controlling intracellular calcium dynamics, and an understanding of this receptor is necessary for an understanding of the control of gene expression, secretion, muscle contraction and many other processes controlled by calcium. IP_3-induced Ca^{2+}-signaling comprises the structural levels channel molecule IP_3R, channel cluster and cluster array on cell level. We investigated behavior on all 3 levels. Based on single-channel data from the type-1 inositol trisphosphate receptor, we showed that the most complex time-dependent model that can be unambiguously determined from steady-state data is one with three closed states and one open state (1). Because the transitions between these states are complex functions of calcium concentration, each model state must correspond to a group of physical states. We found that the main effect of $[Ca^{2+}]$ is to modulate the probability that the receptor is in a state that is able to open, rather than to modulate the transition rate to the open state.

Another study to which we provided the simulations deals with the cluster level (2). It showed that low concentrations of IP_3 cause IP_3Rs to aggregate rapidly and reversibly into small clusters of about four closely associated IP_3Rs. At resting cytosolic $[Ca^{2+}]$, clustered IP_3Rs open independently, but with lower open probability, shorter open time, and less IP_3 sensitivity than lone IP_3Rs. Increasing cytosolic $[Ca^{2+}]$ reverses the inhibition caused by clustering, IP_3R gating becomes coupled, and the duration of multiple openings is prolonged. Clustering both exposes IP_3Rs to local Ca^{2+} rises and increases the effects of Ca^{2+}. Dynamic regulation of clustering by IP_3 retunes IP_3R sensitivity to IP_3 and Ca^{2+}, facilitating hierarchical recruitment of the elementary events that underlie all IP_3-evoked Ca^{2+} signals.

Two studies deal with the cellular level. Our group had predicted in 2003/2004 on theoretical grounds, that Ca^{2+} spiking exhibits a random sequence of interspike intervals instead of a regular oscillation and that the average length of interspike intervals and their standard deviation will sensitively depend on the cytosolic Ca^{2+} buffering capacity. That was verified experimentally for spontaneous spiking in astrocytes, microglia and PLA cells and also for stimulated spiking in HEK cells (5). The effect of Ca^{2+} buffers on individual spikes was investigated in a pure simulation study in collaboration with I.Parker's experimental group (4). It provided new explanations for previously measured time courses of fluorescence signals upon photo-uncaging of IP_3. There is a low cluster density regime and a high cluster density



Simulation of line-scan images of Ca^{2+} release upon a step increase of IP3 in Xenopus oocytes. Fluorescence increases with the cytosolic free [Ca^{2+}]. The color indicates low fluorescence with blue and high fluorescence with red. The control simulation uses 40 µM fluorescent dye as the only exogenous Ca^{2+} buffer and the other panels additionally the indicated amount of slow EGTA or fast BAPTA. We see the typical shortening of the fluorescence signal by competition between EGTA and the dye and the prolongation of release by BAPTA.

regime. In the high density regime, buffers with slow binding rates like EGTA and a K_D in the order of magnitude of cytosolic Ca^{2+} resting levels shape the time course of fluorescence signals by buffer competition but do not shape release at IP_3R clusters. Buffers with fast binding rates shape release by delaying Ca^{2+}-dependent inhibition of IP_3Rs. That causes larger spikes of the number of open channels and prolongs release. The low density regime comprises the effect on spike sequences described above and termination of repetitive spiking by decoupling of clusters by large [EGTA] or [BAPTA].

Our modeling of actin based cell motility aims at suggesting mechanisms which explain the velocity dynamics observed with bacterial propulsion, the morphodynamic types of protrusions observed at the leading edge of a variety of motile cells and to link the types to the state of signaling pathways. The similarity of the molecular components involved in both groups of processes suggests it to be possible to describe both bacterial propulsion and the dynamics of protrusion by models which are distinguished essentially only by parameter values but consist otherwise of very similar equations. We were able to derive such a model from a hypothesis on the relevant processes and to demonstrate the existence of the observed dynamic regimes like steady and oscillatory movement. The research of the group will now establish the link between cell signaling and morphodynamic types.

We are also developing a model of cardiac myocytes. It models individual L-type and ryanodine receptor channels with stochastic state transitions and will still be able to simulate cellular dynamics because of the use of multiscale techniques. The goal of this modeling effort is to simulate excitation contraction coupling with realistic intracellular Ca^{2+} and membrane potential dynamics. The model will serve to investigate conditions and dynamics of Ca^{2+} alternans and membrane potential alternans in first studies. It will then be adapted and enhanced according to the needs of experimental research at the MDC.

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Enculescu, M, Gholami, A, Falcke, M. (2008). Dynamic regimes and bifurcations in a model of actin based motility. Physical Review E 78, 031915

Gunnar Dittmar

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Technical Assistants
Mijo Stanic
Start of group: January 2008

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. We are working closely with these groups to optimize the methods for the different projects.

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 multiple reaction monitoring (MRM). 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).

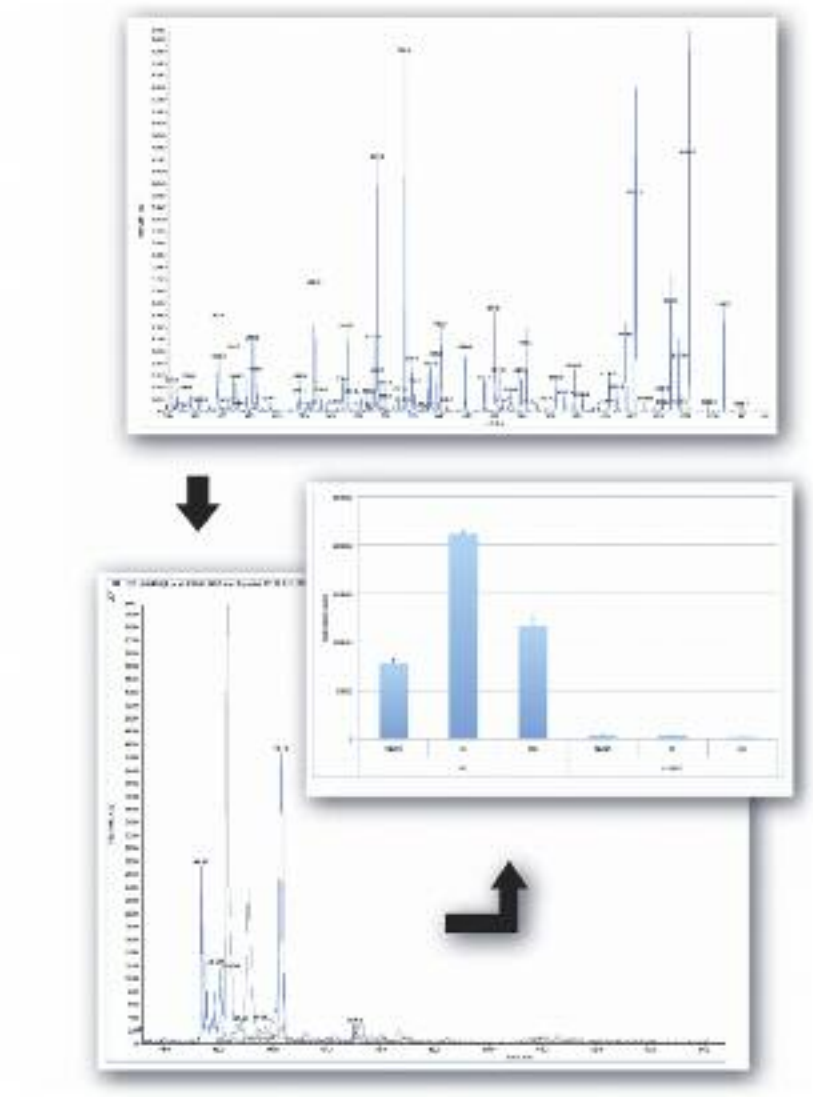
Non-targeted proteomic approaches

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. 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 facility for the quantification of immuno-precipitations and large scale protein identifications.

For samples, which cannot be cultured in media containing heavy amino acids, another technique is available. This technique relies on a highly reproducible liquid chromatography, since two different chromatographic runs have to match. Measurements of non-labeled samples are carried out on a Q-TOF premier as a MS^E experiment.

Identification of post-translational modifications

Besides the translational regulation of proteins, another layer of regulation exists, which is mediated by post-



Development of an MRM method for quantification of peptides.

Starting from the initial MS/MS spectrum of the peptide of interest the MRM-transitions are constructed. Elution profile of different peptides deducted from MRM measurements, which lead to the quantification of the peptide of interest.

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. For the identification of phosphorylation sites the core facility now provides specialized methods, e.g. phospho-ion scan with polarity switching for improved sensitivity.

Selected Pulications

Dittmar, G. and Selbach, M. Novel insights into proteomic technologies and their clinical perspective. Genome Med (2009) vol. 1 (5) pp. 53.

Guenther, U.-P., Handoko, L., Varon, R., Stephani, U., Chang-Yong, T., Mendell, J., Lützkendorf, S., Hübner, C., von Au, K., Jablonka, S., Dittmar, G., Heinemann, U., Schütz, A. and Schülke, M. Clinical variability in distal spinal muscular atrophy type 1 (DSMA1): determination of steady-state IGHMBP2 protein levels in five patients with infantile and juvenile disease. J Mol Med (2009) vol. 87 (1) pp. 31-41.

Schwanhäusser, B., Gossen, M., Dittmar, G. and Selbach, M. Global analysis of cellular protein translation by pulsed SILAC. Proteomics (2009) vol. 9 (1) pp. 205-9.

Anje Sporbert

Structure of the Group

Group Leader
Dr. Anje Sporbert
Start of group: March 2008

Confocal and 2-Photon Microscopy

In March 2008, a microscopy core facility was established at the MDC focusing on confocal and 2-Photon fluorescence microscopy. Starting with two confocal laser scanning microscopes in one room the facility provides now an instrumentation of seven different microscopes each optimised for specific applications in three rooms: four confocal laser scanning microscopes with different configurations (one equipped with an 2-Photon laser), one wide-field fluorescence microscope with two lasers for TIRF-Illumination, one multifocal-2-Photon laser scanning microscope and one wide-field fluorescence microscope with UV-laser for laser-assisted micro-dissection and catapulting. All microscopes are centrally located in rooms equipped with a sterile bench, a CO_2-cell incubator, a small lab bench and gas supplies for the microscope stage incubators offering an ideal environment also for live specimen experiments.

The aim of the Microscopy Core Facility (MCF) is to provide researchers at the MDC a choice of high-end fluorescence microscopes and the necessary support to enable them to perform advanced imaging experiments with different specimens ranging from fixed cells and tissue sections to live cells and organisms.

Potential applications

In confocal microscopy lasers are scanned line-wise over the sample to excite the fluorophores in the specimen. The emitted fluorescence is detected with a digital device via a pinhole in front of it. This pinhole restricts the detected fluorescence to light originating only from the focal plane. The benefits of this technique are (i) a better separation of spectrally overlapping fluorophores as often used in multi-colour fluorescence labellings in many immuno-fluorescence techniques and (ii) a higher axial resolution by the optical z- sectioning effect which is especially prominent in thicker specimen.

The confocal microscopes in the MCF are equipped with different sets of lasers and objectives to offer optimal conditions for multi-colour fluorescence imaging of structures ranging from the subcellular level to the morphology of small organisms (see Figure A/B).

Stage incubators on three of the four confocal microscopes allow for live specimen imaging by controlling the temperature and CO_2-environment. This is a prerequisite for short and long time-lapse experiments to observe, i.e. the development of zebrafish/mouse embryos (Figure B), the migration of cells, the movement of organelles (Figure C) or the different stages of the cell cycle.

The possibility to direct the laser beam to a defined position and illuminate only this area in the sample together with the development of fluorescent proteins with new properties opens up a range of exciting experimental tools for live specimen at the confocal microscopes:

a) Photobleaching a specified area in the specimen and observing how fast the fluorescence recovers into that area ("FRAP") gives indications on the

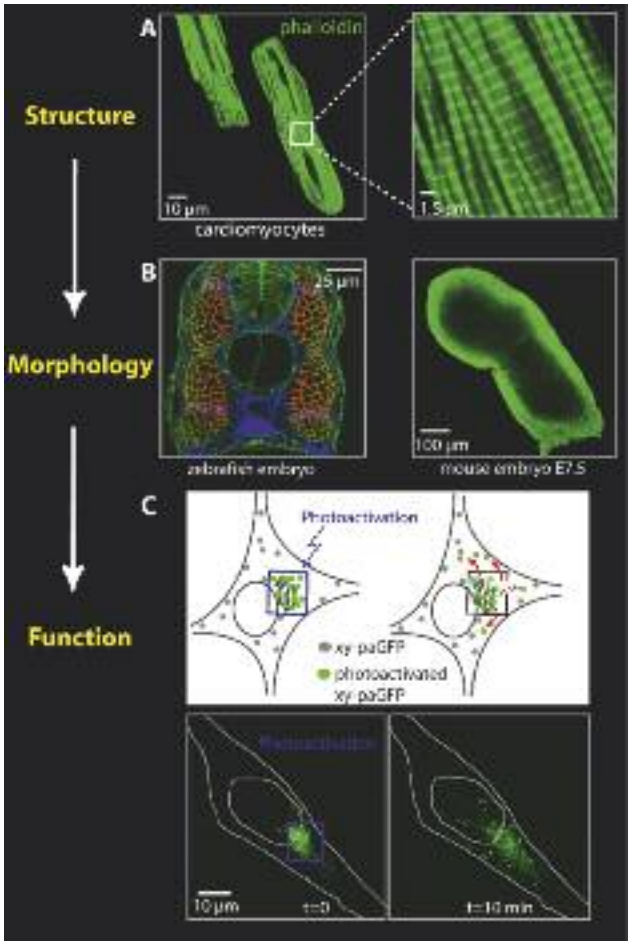
mobility or turnover of the underlying biological structures.

b) Photoactivation or photoswitching of fluorescent proteins (such as paGFP, Dendra, EosFP) in a defined area of the specimen allows for the highlighting of a subset of cells or a fraction of the protein of interest and to follow its migration or movement over time (see Figure C).

In 2-Photon microscopy a pulsed IR-laser is used to excite the fluorophore at about half the normal wavelength (2-Photon effect). This IR light can penetrate deeper into biological samples and is less harmful for it. Therefore, 2-Photon microscopy is especially beneficial for imaging of thicker specimen as mouse embryos, zebrafish embryos, brain sections, tissue preparations. The fixed stage setup at the multifocal-2-Photon microscope allows even for the intravital imaging of, i.e. trafficking cells of the lymphatic or vascular system or the migration of cells into certain tissues.

TIRF-(Total Internal Reflection Fluorescence) Microscopy is an optical technique for the selective imaging of cell membranes or structures in its vicinity in live cells since only fluorophores close to the coverslip (penetration depth of 100-700nm) are excited. TIRF cannot image deep into a specimen. Therefore, it is an ideal technique to observe dynamic processes associated with the cell membrane or the cell surface, i.e. the release and transport of vesicles, cell adhesion molecules and membrane proteins, endocytic and exocytic processes, receptor-ligand interactions.

The Laser microdissection microscope uses the energy of a pulsed UV-laser to cut out selected cells or tissue areas and lifting them up (catapulting) to capture it in a collection device. Parts of chromosomes, individual live or fixed cells, parts of tissue sections or small organisms can be isolated with this technique and processed further, i.e. by recultivating isolated cells/ cell clusters, extracting DNA or RNA. The MCF also offers guidance to find the optimal conditions for the intended experiments (i.e., selection of optimal fluorophores, correct specimen preparation) and help with the following image processing and image analysis.



From subcellular structures to the morphology of small organisms, from the localisation of proteins to the mobility and function of proteins.

***A**: F-actin staining displaying the structure of M-band and Z-disk in fixed rat cardiomyocytes.*

***B**: cross section of stained zebrafish embryo trunk (left, courtesy of C. Otten/S. Seyfried) and whole mount mouse embryo (right, courtesy of F. Spagnoli).*

***C**: live CHO cell expressing APP-paGFP after perinuclear photoactivation showing APP vesicle trafficking.*

Selected Publications

*Görisch SM, *Sporbert A, Stear JH, Grunewald I, Nowak D, Warbrick E, Leonhardt H, Cardoso MC (2008). Uncoupling the replication machinery: replication fork progression in the absence of processive DNA synthesis. Cell Cycle.7,1983-90. * equal contribution

Schmidt V, Sporbert A, Rohe M, Reimer T, Rehm A, Andersen OM, Willnow TE. (2007) SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1. J Biol Chem., 282, 32956-64.

Sporbert A, Domaing P, Leonhardt H, Cardoso MC (2005). PCNA acts as a stationary loading platform for transiently interacting Okazaki fragment maturation proteins. Nucleic Acids Res., 33, 3521-8.

Sporbert A, Gahl A, Ankerhold R, Leonhardt H, Cardoso MC (2002). DNA polymerase clamp shows little turnover at established replication sites but sequential de novo assembly at adjacent origin clusters. Mol Cell, 10, 1355-65.

Hans-Peter Rahn

Structure of the Group

Group Leader
Hans-Peter Rahn

Student Assistant
Jens Hartwig

Secretariat
Daniela Keyner
Start of group: January 2009

Preparative Flowcytometry

Fluorescence-activated cell sorting (FACS) is a specialized type of flowcytometry. It was designed in 1971 by Len Herzenberg to provide a fast physical separation method for fractionating a heterogeneous mixtures of cells into distinct subsets, based upon the light scattering and fluorescent characteristics of different cell types.

The FACS Core Facility assists researchers at the MDC with two state-of-the-art, digital high-speed "FACSAria" sorters and one analog high-speed "FACSVantage SE" sorter from BD. The facility has been used by more than 30 scientific groups at the MDC. Sorted cells are often used for microarray- or sequencing-based gene expression analysis, quantitative real-time PCR, DNA sequencing, live cell imaging or adoptive transfer experiments in animals.

Recent Projects:

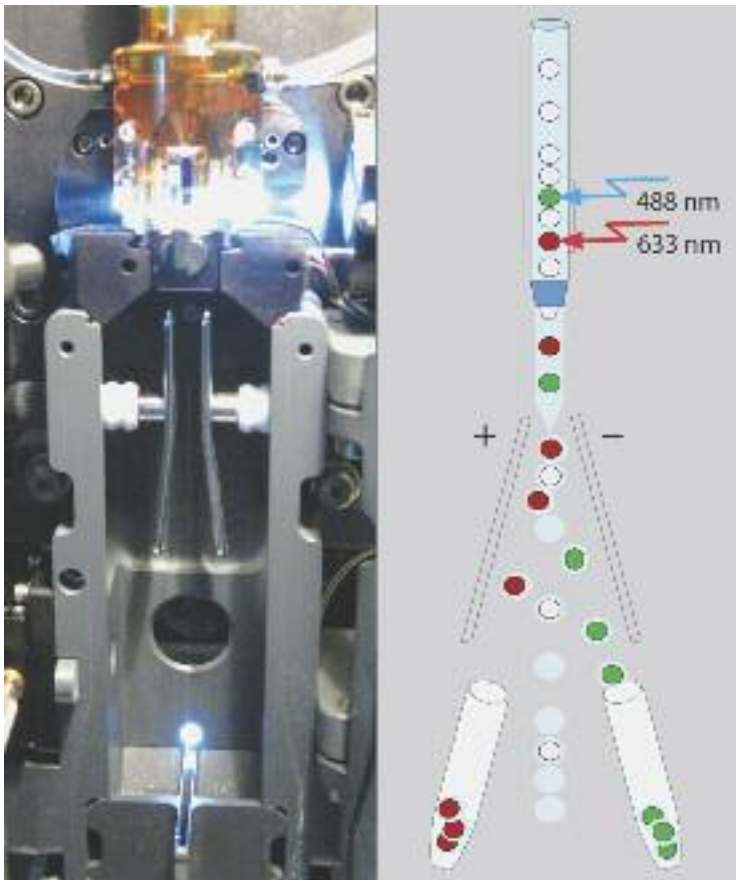
Sophisticated FACS applications are used in the research group of Frank Rosenbauer, with the theme "Cancer, Stem Cells, and Transcription Factors". The interest of this group is to integrate hematopoitic stem cell characteristics with underlying genetic and epigenetic regulatory mechanisms. To identify specific differentiation states of hematopoetic and leukemic cells, the group developed new staining methods using up to nine colors. Routinely, seven color sorts of mouse bone marrow cells are used to analyse hematopoetic cells in vivo and in vitro.

The "Molecular Tumor Genetics and Immunogenetics" group of Martin Lipp investigates the function of T- and B- cells during the development of acute and chronic inflammation. The group focuses on distinct memory and effector T cell populations which are important for the development of antibody-based adaptive immune responses. In this connection central memory, effector/memory und follicular B helper T cells are enriched by FACS using specific patterns of surface markers on these cells.

The "Hematoloy, Oncology and Tumorimmunology" group of Bernd Dörken "Hematoloy, Oncology and Tumorimmunology" uses FACS enrichment of tumor and primary murine hematopoetic cells in order to analyse the function of ectopically expressed genes in comparison to the endogenous expression levels in lymphoma cells.

Together with the research group of Manfred Gossen, FACS was used to create long term stable transgenic cell clones, without the influence of additional anitbiotic resistance genes.

To study early embryogenesis employing high-throughput genomics as well as biochemistry assays, the "Systems Biology" lab of Nikolaus Rajewsky devised a new 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 2 (flow cell with sort block and deflection plates),

Right: Schematic view of the separation principle

Selected publications

Broske, A.M., Vockentanz, L., Kharazi, S., Huska, M.R., Mancini, E., Scheller, M., Kuhl, C., Enns, A., Prinz, M., Jaenisch, R., et al. (2009). DNA methylation protects hematopoietic stem cell multipotency from myeloerythroid restriction. Nat Genet 41, 1207-1215.

Rasheed, A.U., Rahn, H.P., Sallusto, F., Lipp, M., and Muller, G. (2006). Follicular B helper T cell activity is confined to CXCR5(hi)ICOS(hi) CD4 T cells and is independent of CD57 expression. Eur J Immunol 36, 1892-1903.

Mathas, S., Kreher, S., Meaburn, K.J., Johrens, K., Lamprecht, B., Assaf, C., Sterry, W., Kadin, M.E., Daibata, M., Joos, S., et al. (2009). Gene deregulation and spatial genome reorganization near breakpoints prior to formation of translocations in anaplastic large cell lymphoma. Proc Natl Acad Sci U S A 106, 5831-5836.

Kaufman, W.L., Kocman, I., Agrawal, V., Rahn, H.P., Besser, D., and Gossen, M. (2008). Homogeneity and persistence of transgene expression by omitting antibiotic selection in cell line isolation. Nucleic Acids Res 36, e111.

Stoeckius, M., Maaskola, J., Colombo, T., Rahn, H.P., Friedlander, M.R., Li, N., Chen, W., Piano, F., and Rajewsky, N. (2009). Large-scale sorting of C. elegans embryos reveals the dynamics of small RNA expression. Nat Methods 6, 745-751.

Bettina Erdmann

Structure of the Group

Group Leader
Dr. Bettina Erdmann

Technical Assistants
Marianne Vannauer
Margit Vogel (part-time)

Electron Microscopy

Methods of ultrastructural research are increasingly being used again to characterize genetically modified specimens. During the last years, the electron microscopy (EM) facility has established and extended a panel of approaches, protocols and the technical basis for morphological studies for a wide range of research projects. Based on two transmission electron microscopes (Zeiss 910, FEI Morgagni), both equipped with high resolution CCD-cameras, we offer conventional plastic embedding for phenotyping, immunogold labeling with cryo methods as well as negative staining. In this way, collaborations with more than 25 research groups in the house were performed, using the major model systems like cultured cells, mouse, yeast, zebrafish and biopsie probes from clinical projects. Only some typical examples for EM service are mentioned here:

Phenotyping/ultrastructural characterization

By using Lumox dishes for cell growth, we were able to retain the spatial arrangement of cultured cells during EM processing. With this approach one can easily identify differentiation of stem cells even at the light miscroscopical level (Fig. a, collaboration with K. Eckert). At higher magnifications we could detect cellular components like myofibrils and desmosomes in multilayered cell cultures of cardiomyocytes (Fig. b, collaborations with B. Gerull and C. Özcelik). Furthermore, we found a widened endoplasmic reticulum and signs of stress in glioma cell cultures (collaboration with H. Kettenmann). Fibroblast cell lines with different oncogenes revealed remarkable changes in the number of autophagosomes (collaboration with C. Schmitt).

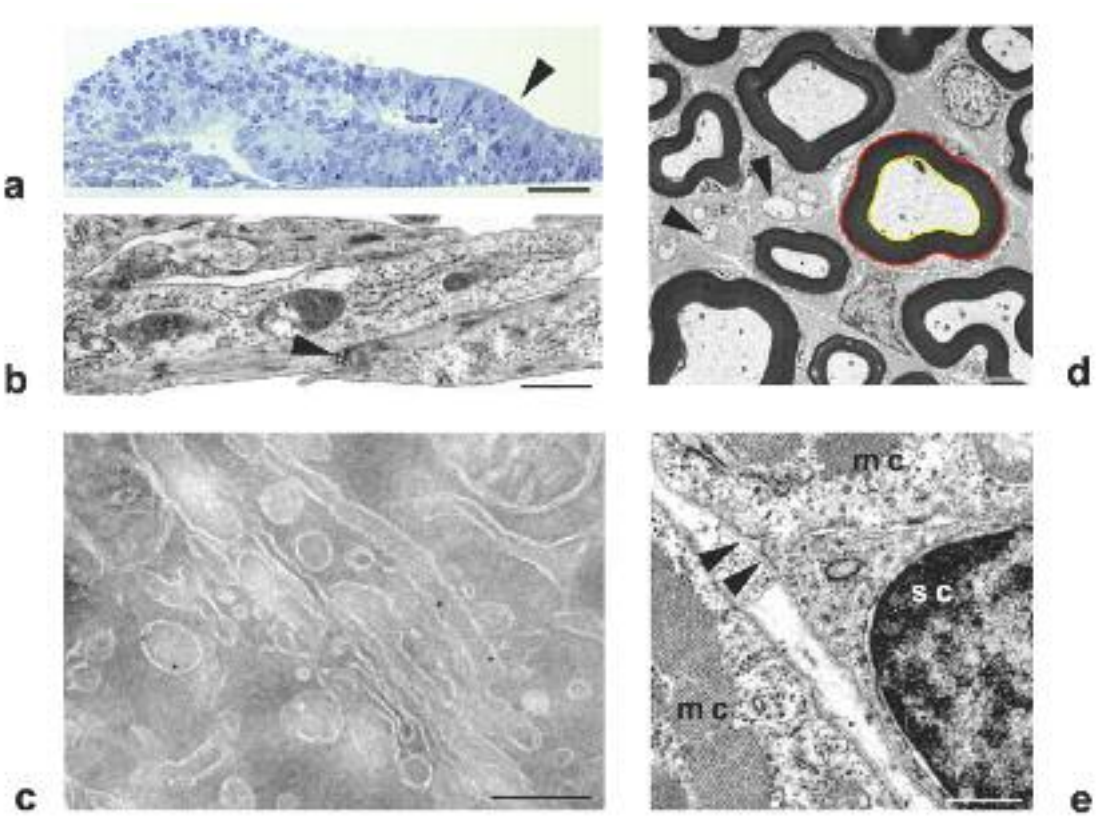
A range of research projects to `Function and Dysfunction of the Nervous System` analyze model organisms with defects in nerve fibers and myelin structure. Together with C. Birchmeier, G. Lewin and A. Garratt a useful approach was developed to determine the number of axons in peripheral nerves and the degree of myelination (*g-ratio*). A lot of phenotypes of mice and mole rats were described in this way (Fig. d), and therefore we recently try to establish a new software module for the automatical detection of myelin figures.

Other types of phenotyping at the ultrastructural level reveal details that cannot be resolved by fluorescence labeling and confocal microscopy. We identified and counted the number of satellite cells in the developing muscle in different mutant mice (Fig. e, collaboration with C. Birchmeier). In contrast to fibroblasts or endothelial cells in this tissue, these satellite cells are located under the basal membrane close to the muscle cells.

Immunocytochemical labeling

The available expertise in the use of cryosectioning techniques according to Tokuyasu was extended and increasingly applied for immunolabeling of tissues. Although it often remains difficult to find an acceptable compromise between fixation, structure preservation and the retaining of antigenicity, in some cases these methods yield excellent results even in complex tissue like brain. So we could detect a reduced number of amyloid precursor protein (APP) molecules in Golgi fields in the hippocampus of *Sorla*-deficient mice (Fig. c, collaboration with T. Willnow). Using Lamp1 as marker, we quantified with the same method lysosomal changes in kidneys of chloride channel knock out mice (collaboration with T. Jentsch). In peroxisomes of the

Examples fort the use of EM methods for phenotyping and immunolabeling



a. cell culture of human embryonic stem cells, line SA 002, embedding in Epon as for EM, semithin section (1µm), light microscopy, bar = 50µm

a spontaneous differentiation of cells towards high prismatic, epithelial-like structures becomes visible (arrowhead)

b.cell culture of neonatal rat cardiomyocytes, embedding in Epon, ultrathin section, bar = 1µm

cell components like growing myofibrils and cell-cell-contacts (arrowhead) are detectable

c. hippocampus of an adult mouse, ultrathin cryosection according to Tokuyasu, bar = 250 nm

immunolabeling of amyloid precursor protein (APP) in Golgi fields, 12 nm colloidal gold

d. saphenous nerve of a mole rat, embedding in Epon, ultrathin section, bar = 2µm

the number of myelinated and unmyelinated (arrowheads) axons can be counted, and g-ratios can be calculated from myelin and axon perimeters (coulored lines)

e. limb muscle of a mouse embryo, embedding in Epon, ultrathin section, bar = 500 nm

identification of satellite cells (sc) among muscle cells (mc) and other cell types is done finally by electron microscopy, as depicted by the basal membrane covering both cell types (arrowheads)

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heart, we firstly localized the soluble epoxide hydroxylase (sEH), an enzyme with putative importance to prevent heart failure (collaboration with W.-H. Schunck).

Selected publications

Wetzel, C, Hu, J, Riethmacher, D, Benckendorff, A, Harder, L, Eilers, A, Moshourab, R, Kozlenkov, A, Labuz, D, Caspani, O, Erdmann, B, Machelska, H, Heppenstall, PA, Lewin, GR. (2007). A stomatin-domain protein essential for touch sensation in the mouse. Nature 445, 206-209.

Vasyutina, E, Lenhard, DC, Wende, H, Erdmann, B, Epstein, JA, Birchmeier, C. (2007). RBP-J (Rbpsuh) is essential to maintain muscle progenitor cells and to generate satellite cells. PNAS 104, 4443-4448

Rohe, M, Carlo, A-S, Breyhan, H, Sporbert, A, Militz, D, Schmidt, V, Wozny, C, Harmeier, A, Erdmann, B, Bales, KR, Wolf, S, Kempermann, G, Paul, SM, Schmitz, D, Bayer, TA, Willnow, TE, Andersen, OM. (2008) Sortilin-related receptor with A-type repeats (SORLA) affects the amyloid precursor protein-dependent stimulation of ERK signaling and adult neurogenesis. J Biol. Chem. 283, 14826-14834

König, O, Rüttiger, L, Müller, M, Zimmermann, U, Erdmann, B, Kalbacher, H, Gross, M, Knipper, M. (2008) Estrogen and the inner ear: Megalin knockout mice suffer progressive hearing loss. FASEB J. 22, 410-417

Gramlich, M, Michely, B, Krohne, C, Heuser, A, Erdmann, B, Klaassen, S, Hudson, B, Magarin, M, Kirchner, F, Todiras, M, Granzier, H, Labeit, S, Thierfelder, L, Gerull, B. (2009, 47, 352-358) Stress-induced dilated cardiomyopathy in a knock-in mouse model mimicking human titin-based disease. J Mol Cell Cardiol 47, 352-358

Boris Jerchow

Structure of the Group

Group Leader
Dr. Boris Jerchow

Secretariat
Christine Krause

Technical Assistants
Katja Becker
Tianwu Qiao
Andrea Leschke

Transgenics

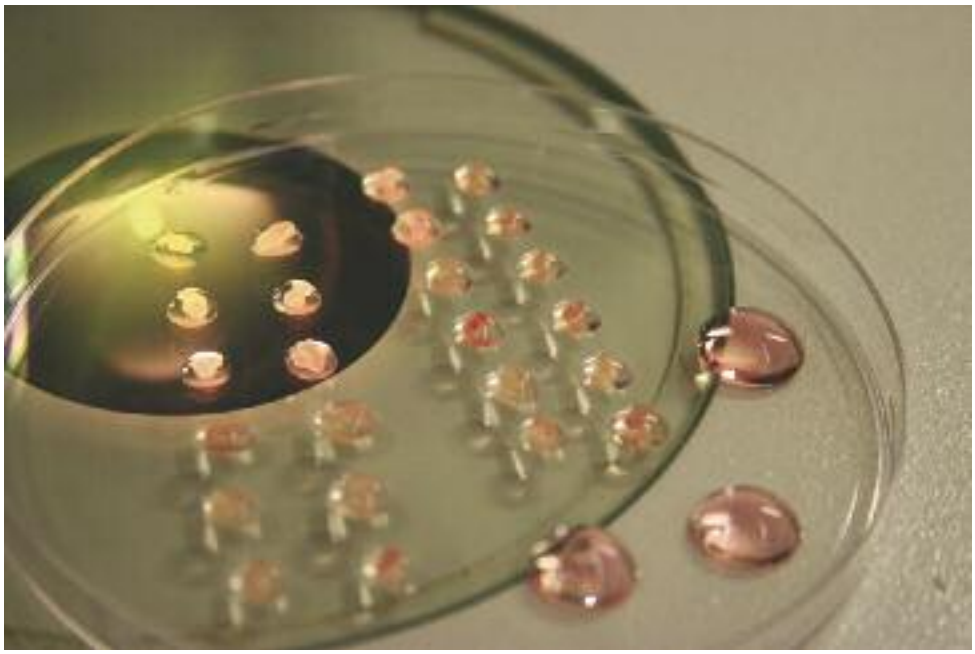
The Transgenic Core Facility (TCF) offers expertise in all kinds of mouse Assisted Reproductive Technologies (ART). Starting at the planning phase, we support scientists from the MDC and their external collaborators with the design and layout of their projects. One focus of our work is the generation 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 microinjection of plasmid or BAC type transgenes into the pronuclei of fertilized oocytes. During the last year, we had the chance to develop a related technique together with Lajos Mates from the group of Zsuzsanna Izsvak: After coinjection of a transgene flanked by specific inverted repeats together with RNA encoding an optimized version of the Sleeping Beauty Transposase, SB100, into oocytes, an enzyme-mediated integration of the transgene takes place (refer to selected publication). Compared to conventional microinjection this technique leads to the integration of single transgenes instead of the integration of an uncontrollable number of copies as a concatamer and yields a higher rate of transgenic founders. The size of the transgene is a limitation of this technique, though, and only plasmid type sized transgenes can be integrated into the genome.

A second focus of our work is the conservation of precious mouse lines and the rederivation of conserved lines. This service has been continuously expanded during the last years both in quantity and in terms of the different protocols that have been optimized in the lab. We can now offer the long term cryopreservation of pre-implantation embryos as well as sperm 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. We therefore see an increased demand for revitalization of lines conserved by third parties following a variety of protocols. In this context we were successful to establish a new protocol developed by the Jackson Laboratories that make even recalcitrant lines like C57BL/6 amenable for *in vitro* fertilization. In addition, we have a laser device at our disposal that can be used to penetrate the outer *zona pellucida* of the oocyte to facilitate sperm entry and thereby achieve fertilization even in cases, where this does not spontaneously happen.

In addition to the above, we have just taken on the rederivation of hygienically compromised lines by embryo transfer. Rederivation by hysterectomy is therefore discontinued.

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 more.

Besides ongoing projects for our clients, the current center of our work is the optimization of ES cell deriva-



Oviducts lined up in pre-implantation embryo culture medium before zygote collection.

tion and culture conditions and the work with C57BL/6 derived ES cell lines, namely the generation of coat color chimeras with these lines. Furthermore, we aim at implicating additional steps in the quality control of materials received before employing them in the generation of genetically modified subjects. By these means we hope to achieve a refinement that will not only improve our service but also reduce cost and at the same time reduce the number of animals we need to complete a project.

Record 2008:

- 18 lines conserved
- 32 lines from recombinant ES cells
- 12 transgenic lines
- 1 recombinant ES cell line
- 1 conserved line (third party) rederived

Selected publication

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 Genetics 41, 753-761.

Academics

Akademische Aktivitäten

Academic Appointments 2008-2009

Berufungen 2008-2009

Appointments at the MDC/Joint Appointments

The MDC has established an official cooperation agreement with the Humboldt University Berlin (HUB) and the Free University of Berlin (HUB) which permits joint appointments. Many of the scientists appointed to the MDC are interested in a joint appointment with one of the universities of Berlin. Through this academic link, they wish to participate actively in teaching as well as ensure access to the Berlin universities for their Masters and PhD students. The MDC and the Berlin universities, likewise, open up the possibility to their employees to do doctoral studies and to qualify as lecturers and professors in the corresponding Faculties.

Since 2002, when the 5th Amending Act of the Framework Law Governing Universities (*Hochschulrahmengesetz*) went into effect, the MDC has been able to appoint junior professors jointly with the Berlin Universities. In respect to the collaboration between the MDC and the HUB in 1994, a Supplementary Agreement was concluded in 2002 which allows for the appointment of junior professors similar to the guidelines for joint appointments to conventional professorships. Likewise, the MDC signed a Supplementary Agreement with the FUB in December 2002.

In June 2003, the Charité – Universitätsmedizin Berlin became the common medical faculty of both the FUB and HUB and, since then, most of the appointments at the MDC have been done in cooperation with the Charité, reflecting the close connection between the two establishments. Currently, the following joint MDC-Charité positions exist at the MDC: ten W3/C4 Professors, three W2/C3-Professors, and two Junior Professors. In addition, together with the FUB, the MDC has appointed two W3/C4-Professors and, with the HUB two W3/C4-Professors. Furthermore, 12 appointed scientists (W3/C4 und W2/C3) from the Charité and one scientist (W3) from the HUB are simultaneously group leaders at the MDC.

2008

MDC Research Group Leader **Dr. M. Cristina Cardoso** has accepted an appointment to become a W3 professor of molecular cell biology in the biology department of the Technical University Darmstadt. There the Lisbon-born cell biologist will intensify her research on the regulation,

Berufungen an das MDC/Gemeinsame Berufungen

Das MDC hat mit der Humboldt-Universität zu Berlin (HUB) und der Freien Universität Berlin (FUB) Kooperationsverträge abgeschlossen, die gemeinsame Berufungen erlauben. Viele der an das MDC berufenen Wissenschaftlerinnen und Wissenschaftler sind an einer gemeinsamen Berufung mit einer der Berliner Universitäten interessiert. Sie möchten durch diese akademische Anbindung aktiv an der Lehre teilnehmen, den Zugang für ihre Diplomanden/innen bzw. Doktoranden/innen zu den Berliner Universitäten sichern und ihren Mitarbeiterinnen und Mitarbeitern die Möglichkeit eröffnen, sich an den entsprechenden Fakultäten zu promovieren und zu habilitieren.

Seit 2002 kann das MDC, auf Grundlage des 5. Änderungsgesetzes zum Hochschulrahmengesetz, gemeinsam mit Berliner Universitäten Junior Professoren berufen. Auf der Grundlage der Vereinbarung über die Zusammenarbeit zwischen dem MDC und der HUB zu Berlin aus dem Jahre 1994 wurde 2002 eine Ergänzungsvereinbarung abgeschlossen, die die Ernennung von Junior-Professoren analog der Leitsätze für gemeinsame Berufungen auf konventionelle Professuren ermöglicht. Das MDC hat im Dezember 2002 in gleicher Weise mit der FUB eine Ergänzungsvereinbarung beschlossen.

Seit der Gründung der Charité – Universitätsmedizin Berlin als gemeinsame Medizinische Fakultät der FUB und der HUB im Juni 2003 erfolgen aufgrund der engen Kooperation von MDC und Charité die meisten Berufungen als gemeinsame Berufungen mit der Charité. Zur Zeit arbeiten am MDC insgesamt zehn gemeinsam mit der Charité berufene W3/C4-Professoren, drei W2/C3-Professoren und zwei Juniorprofessoren, zwei gemeinsam mit der FUB berufene W3/C4-Professoren und zwei gemeinsam mit der HUB berufene W3/C4-Professoren und ein W2-Professor. Darüber hinaus sind 12 berufene Wissenschaftler (W3/C4 und W2/C3) der Charité und ein berufener Wissenschaftler (W3) der HUB gleichzeitig Forschungsgruppenleiter am MDC.

2008

*MDC-Forschungsgruppenleiterin **Dr. M. Cristina Cardoso** hat einen Ruf auf eine W3-Professur für Molekulare Zellbiologie im Fachbereich Biologie der Technischen Universität Darmstadt angenommen. Dort wird die aus Lissabon stammende Zellbiologin ihre Forschung über die Regulati-*

replication and reprogramming of the epigenome, which is of special significance for cancer research. In 1995 Dr. Cardoso came from Harvard Medical School (Boston, USA) to Berlin-Buch as research group leader at the Franz Volhard Clinic of the Charité; in 1997 she was appointed research group leader at the MDC.

The biophysicist **Dr. Jing Hu** (MDC) has accepted the position of junior group leader at the Werner Reichardt Centre for Integrative Neuroscience in Tübingen. There she continues to work on the molecular and cellular mechanisms of mechanosensation and pain. Dr. Hu came to the MDC in 2001 as Alexander von Humboldt Research Fellow in the research group of **Professor Gary Lewin**, after completing her PhD at the Institute of Biophysics of the Chinese Academy of Sciences in Beijing. 2008 she received a Max Delbrück Scholarship (formerly named Helmholtz Scholarship) of the MDC for her achievements in the field of pain research.

The director of the Franz Volhard Center for Cardiovascular Diseases and Nephrology of the Charité, Campus Berlin, **Professor Jens Jordan,** was appointed director of the Department of Clinical Pharmacology at the Hannover Medical School in April 2008. Professor Jordan was a Helmholtz fellow at MDC before he accepted the new position at the Volhard Clinic in 2002. Since that year he is also extraordinary member of the Drug Commission of German Physicians.

Professor Helmut Kettenmann (MDC) is the new president of the Federation of the European Neurosciences Societies. He took over the position at the annual meeting of FENS on July 16, 2008 in Geneva (Switzerland) from **Professor Richard Morris** (University of Edinburgh, UK), who had held the position since 2006. The FENS was founded in Berlin in 1998. It represents 28 neuroscience societies in Europe and has about 16,000 members.

Since July 1, 2008, **Dr. Olaf Rötzschke** (MDC) has been heading an independent research group at the "Singapore Immunology Network (SIgN)". The recently opened institute is the youngest spin-off of the international "Biopolis" research campus in Singapore, which focuses on basic research and different aspects of translational research in the life sciences.

Biochemist **Dr. Björn Christian Schroeder** from the University of California, San Francisco, USA, has been appointed as a junior group leader at the MDC. In November 2008, he started his research group in Berlin-

on, Replikation und Reprogrammierung des Epigenoms, die von besonderer Bedeutung für die Krebsforschung ist, weiter führen. Dr. Cardoso kam 1995 von der Harvard Medical School (Boston, USA) als Gruppenleiterin an die Franz-Volhard-Klinik der Charité, 1997 hatte sie eine Forschungsgruppe am MDC erhalten.

*Die Biophysikerin **Dr. Jing Hu** (MDC) hat den Ruf auf eine Juniorgruppenleiterstelle am Werner Reichardt Centre for Intergrative Neuroscience (CIN) in Tübingen angenommen. Sie erforscht dort die molekularen und zellulären Mechanismen von Berührung und Schmerz. Dr. Hu war nach ihrer Promotion in Biophysik an der Chinesischen Akademie der Wissenschaften in Peking 2001 als Alexander von Humboldt Research Fellow an das MDC in die Forschungsgruppe von **Prof. Gary Lewin** gekommen. 2008 hatte sie ein Max-Delbrück-Stipendium (vormals Helmholtz-Stipendium) des MDC erhalten.*

*Der Leiter des Franz-Volhard-Zentrums für Herzkreislauf-Erkrankungen, Nephrologie der Charité, Campus Berlin-Buch, **Prof. Jens Jordan,** ist im April 2008 als Direktor der Abteilung für Klinische Pharmakologie an die Medizinische Hochschule Hannover berufen worden. Er war am MDC Helmholtz-Fellow, bevor er 2002 die Aufgabe in der Volhard-Klinik übernahm. Seit 2002 ist er zudem außerordentliches Mitglied der Arzneimittelkommission der Deutschen Ärzteschaft.*

***Prof. Helmut Kettenmann** (MDC) ist neuer Präsident der Föderation der Europäischen Neurowissenschaftlichen Gesellschaften (FENS). Er übernahm das Amt auf der Jahrestagung der FENS am 16. Juli 2008 in Genf (Schweiz) von **Prof. Richard Morris** (Universität Edinburgh, Großbritannien), der die Position seit 2006 innehatte. Die FENS wurde 1998 in Berlin gegründet. Sie vertritt 28 neurowissenschaftliche Fachgesellschaften Europas und hat rund 16.000 Mitglieder.*

***Dr. Olaf Rötzschke** (MDC) leitet seit 1. Juli 2008 eine unabhängige Forschergruppe am „Singapore Immunology Network (SIgN)". Das 2006 eröffnete Institut ist die jüngste Ausgründung des internationalen „Biopolis" Forschungscampus in Singapur, der sich mit Grundlagenforschung und verschiedenen Aspekten der „translationalen" Forschung auf dem Gebiet der Lebenswissenschaft befasst.*

*Der Biochemiker **Dr. Björn Christian Schroeder** von der University of California, San Francisco, USA, ist als Nachwuchsgruppenleiter an das MDC berufen worden und hat im November 2008 seine Arbeit in Berlin-Buch aufgenom-*

Buch which is part of the Excellence Initiative "NeuroCure". In the USA, Dr. Schroeder discovered and cloned a new family of ion channels. By studying these channels, scientists hope to get new findings about physiological processes in different organs.

The administrative director of the MDC, **Dr. Stefan Schwartze**, has been named new chancellor of the University of Münster. He received his certificate of appointment in Münster on January 3, 2008 after the senate of the university, in an extraordinary session on December 19, 2007, nominated the 41-year-old jurist for appointment by the North Rhine-Westphalian Science Minister. He assumed his new position on February 1, 2008 for an eight-year term of office.

Cell biologist **Professor h. c. Thomas Sommer** (MDC) has accepted the appointment to become full professor (W3) for cellular biochemistry of the Faculty of Mathematics and Natural Sciences at the Humboldt University (HU) Berlin. Since 1999, Professor Sommer has been a research group leader at the MDC. His area of expertise is protein degradation. Since 2004, he has served as Vice Scientific Director of the MDC.

Diabetes researcher **Dr. Francesca Spagnoli** of Rockefeller University in New York, USA, has come as Helmholtz Junior Group Leaders to the MDC and the Charité. Her major field of interest is stem cell research, and she is currently investigating the embryonic development of beta cells in the Langerhans' islets of the pancreas.

Diabetes researcher **Dr. Matthew Poy** of the Swiss Federal Institute of Technology (ETH) Zurich, has come as Helmholtz Junior Group Leaders to the MDC and the Charité. He investigates the processes that lead to the outbreak of diabetes. He was able to show, that microRNAs play a crucial role in the regulation of metabolic processes.

As of August 1, 2008 **Cornelia Lanz** is the new Administrative Director of the MDC. She comes to her new position from Lübeck University of Applied Sciences, where she was chancellor for the last four years until July 31, 2008. At the MDC she succeeds **Dr. Stefan Schwartze**, who was appointed chancellor of the University of Münster.

men. Er ist mit seiner MDC-Forschungsgruppe in die Berliner Exzellenzinitiative NeuroCure eingebunden. In den USA entdeckte und klonierte er eine neue Familie von Ionenkanälen. Von der Analyse dieser Gruppe von Kanälen erhofft sich die Forschung neue Erkenntnisse über physiologische Prozesse in vielen Organen.

Der administrative Vorstand des MDC, ***Dr. Stefan Schwartze****, ist am 3. Janur 2008 zum Kanzler der Westfälischen Wilhelms-Universität Münster ernannt worden. Der Senat der Universität hatte den 41-jährigen Juristen am 19. Dezember 2007 in einer außerordentlichen Sitzung dem nordrhein-westfälischen Wissenschaftsminister zur Ernennung vorgeschlagen. Er trat sein neues Amt zum 1. Februar 2008 an. Seine Amtszeit beträgt acht Jahre. Dr. Schwartze war am 1. August 2003 vom Bundesministerium für Bildung und Forschung (BMBF) als Verwaltungschef an das MDC gekommen.*

Der Zellbiologe ***Prof. h. c. Thomas Sommer*** *(MDC) hat den Ruf auf die W3-Professur für das Fachgebiet „Zelluläre Biochemie" der Mathematisch-Naturwissenschaftlichen Fakultät I der Humboldt-Universität zu Berlin angenommen. Prof. Sommer ist seit 1999 Forschungsgruppenleiter am MDC. Sein Spezialgebiet ist der Proteinabbau. Seit 2004 ist er außerdem stellvertretender wissenschaftlicher MDC-Vorstand.*

Die Diabetesforscherin ***Dr. Francesca Spagnoli*** *von der Rockefeller Universität in New York, USA, ist als Leiterin einer Helmholtz-Nachwuchsforschergruppe an das MDC und die Charité gekommen. Dr. Spagnoli arbeitet auf dem Gebiet der Stammzellforschung und erforscht die Beta-Zellen der Bauchspeicheldrüse.*

Der Diabetesforscher ***Dr. Matthew Poy*** *ist von der Eidgenössischen Technischen Hochschule (ETH) Zürich, Schweiz, als Helmholtz-Nachwuchsforschergruppenleiter an das MDC und die Charité gekommen. Dr. Matthew Poy untersucht die Prozesse, die zur Entstehung von Diabetes führen. Er konnte zeigen, dass kleine microRNAs, kurze Ribonukleinsäurestränge, eine entscheidende Rolle bei der Regulation von Stoffwechselprozessen spielen.*

Die frühere Kanzlerin der Fachhochschule Lübeck, ***Cornelia Lanz****, ist seit 1. August 2008 neuer administrativer Vorstand des MDC. Frau Lanz, die die vergangenen vier Jahre bis zum 31. Juli 2008 in Lübeck arbeitete, tritt die Nachfolge von Dr. Stefan Schwartze an, der zum Kanzler der Universität Münster ernannt worden war.*

2009

Physician **Professor Walter Rosenthal** has been appointed as Scientific Director of the MDC. The former director of the Leibniz-Institut für Molekulare Pharmakologie (FMP) succeeds cancer researcher **Professor Walter Birchmeier** who has served as the MDC Scientific Director for the past five years and who now focuses again to his MDC research group.

At their annual meeting in March, 2009 the members of the German Society for Chrystallography (DGK) elected **Professor Udo Heinemann** to be Chairman of the Board. The term of office is three years. Professor Heinemann succeeds **Professor Wolfgang Neumann** from Humboldt University Berlin. The DGK has more than 1000 members.

In January, **Dr. Wei Chen,** head of the research group Applied Bioinformatics at the Max Planck Institute (MPI) of Molecular Genetics, Berlin, has been appointed to MDC´s Berlin Institute for Medical Systems Biology (BIMSB). Dr. Chen heads the technology platform "Genomics", which uses state-of-the-art parallel sequencing technologies. They are considerably faster and more cost-effective than conventional sequencing equipment. With the new generation of sequencers, Dr. Chen has established a number of different methods to investigate the function of genes

Dr. Markus Landthaler from Rockefeller University in New York, USA, has been appointed to the Berlin Institute for Medical Systems Biology (BIMBS). He began his work as junior research group leader at the MDC at the beginning of March. His research area is concerned with microRNAs and RNA binding proteins, which together are instrumental in regulating protein synthesis in the cells. This regulation takes place in complex networks, the understanding of which can explain the mechanisms of disease pathogenesis and development. For this purpose, Dr. Landthaler has established special RNA-protein cross-linking experiments which he combines with the new technologies in the BIMSB, deep sequencing and mass spectrometry, to elucidate a system-wide under-standing of RNA-based and protein-based post-transcriptional regulation mechanisms.

Dr. Christoph Dieterich of the Max Planck Institute (MPI) of Developmental Biology, Tübingen, came to the Berlin Institute for Medical Systems Biology (BIMSB) of the MDC in April 2009. He heads the technology platform

2009

*Der Pharmakologe **Prof. Walter Rosenthal** und bisherige Direktor des Leibniz-Instituts für Molekulare Pharmakologie (FMP) ist seit Januar 2009 neuer wissenschaftlicher Vorstand des MDC. Er ist Nachfolger von Krebsforscher Prof. Walter Birchmeier, der dieses Amt fünf Jahre inne hatte und sich wieder verstärkt seiner Forschungsgruppe am MDC widmet.*

*Auf ihrer Jahrestagung haben die Mitglieder der Deutschen Gesellschaft für Kristallographie (DGK) am 10. März 2009 **Prof. Udo Heinemann** zum neuen Vorstandsvorsitzenden gewählt. Die Amtsperiode beträgt drei Jahre. Prof. Heinemann löst **Prof. Wolfgang Neumann** von der Humboldt-Universität Berlin ab. Die DGK hat mehr als 1 000 Mitglieder.*

***Dr. Wei Chen**, Leiter der Forschungsgruppe Angewandte Bioinformatik am Max-Planck-Institut (MPI) für Molekulare Genetik, Berlin, ist im Januar 2009 an das Berlin Institute for Medical Systems Biology (BIMSB) des MDC gekommen. Dort leitet er die wissenschaftliche Technologieplattform „Genomics", die modernste, parallel arbeitende Sequenziertechnologien einsetzt, die erheblich schneller und kostengünstiger sind als herkömmliche Sequenzierapparate. Mit den neuen Maschinen hat Dr. Chen im BIMSB eine Reihe verschiedener Ansätze für die Erforschung der Funktion von Genen eingerichtet.*

*Im März 2009 hat der Molekularbiologe **Dr. Markus Landthaler** von der Rockefeller Universität in New York, USA, seine Arbeit als Nachwuchsgruppenleiter am Berlin Institute for Medical Systems Biology (BIMSB) des MDC aufgenommen. Arbeitsgebiet von Dr. Landthaler sind kleine Ribonukleinsäuren (microRNAs) sowie RNA-bindende Proteine, die zusammen maßgeblich die Proteinsynthese in Zellen regulieren. Diese Regulation spielt sich in komplexen Netzwerken ab, deren Verständnis auch die Mechanismen der Entwicklung und Krankheitsentstehung erklären. Er hat hierzu spezielle RNA-Protein-Kreuzvernetzungsexperimente etabliert, die er mit den neuen Technologien im BIMSB, der Tiefensequenzierung und der Massenspektrometrie, kombiniert. Damit will er ein systemweites Verständnis von RNA- und Protein-basierten posttranskriptionalen Regulationsmechanismen erarbeiten.*

***Dr. Christoph Dieterich** vom Max-Planck-Institut (MPI) für Entwicklungsbiologie, Tübingen, ist im April 2009 an das Berlin Institute for Medical Systems Biology (BIMSB) des*

"Bioinformatics in Quantitative Biology". He and his colleagues develop computer methods and strategies to evaluate data from gene analyses, mass spectrometry and imaging procedures. The objective is to achieve a better understanding of biological systems.

Dr. Stefan Kempa from the MPI of Molecular Plant Physiology, Potsdam, came to the Berlin Institute for Medical Systems Biology (BIMSB) of the MDC in April 2009. He heads the technology platform "Integrative Proteomics and Metabolomics". It uses the latest technologies of mass spectrometry to investigate metabolic processes and the proteome in cell cultures, organs, and whole organisms.

The physicist **Professor Thoralf Niendorf** has been appointed to the chair for Experimental Ultra High Field Magnetic Resonance Imaging (MRI) at the Experimental and Clinical Research Center (ECRC) of MDC and Charité. The specialist for imaging tech-niques from RWTH Aachen University became head of the MRI facility of ECRC on Campus Berlin-Buch on August 10, 2009. The research facility, which has one of the world's strongest magnetic resonance tomographs, a 7 Tesla whole-body MRI scanner, was dedicated in January 2009 by Federal Education and Research minister **Annette Schavan**.

The neuroscientist **Dr. James Poulet** has been appointed to the Berlin excellence cluster and as of July 1, 2009 began work there in Berlin-Mitte. Dr. Poulet, whose research is being funded by the MDC, which along with other institutions is participating in NeuroCure, is studying how neuronal regulatory circuits control behavior. Until the summer of 2009 he was a postdoc at École Polytechnique Fédérale de Lausanne, Switzerland, where he succeeded in recording intracellular activity between two nerve cells in the cerebral cortex of a conscious animal for the first time.

Formerly at the Humboldt University of Berlin, **Dr. Jana Wolf** joined the MDC as a junior group leader as part of the Helmholtz Association's initiative "Systems Biology". Dr. Wolf's group will receive additional funding through the BMBF's program "Research Units in Systems Biology (FORSYS) ". Her work focuses on mathematical modeling of cellular processes.

MDC gekommen und baut dort die Technologieplattform ‚Bioinformatik und Quantitative Biologie'auf. Er und seine Mitarbeiter entwickeln Computermethoden und Analysestrategien, um die Daten von Genanalysen, Massenspektrometrie und bildgebenden Verfahren auswerten zu können. Ziel ist ein besseres Verständnis biologischer Systeme.

***Dr. Stefan Kempa** vom MPI für Molekulare Pflanzenphysiologie, Potsdam, ist im April 2009 an das Berlin Institute for Medical Systems Biology (BIMSB) des MDC gekommen und baut dort die Technologieplattform „Integrative Proteomics and Metabolomics" auf. Dort setzt er die neuesten Technologien der Massenspektrometrie ein, um Stoffwechselvorgänge und das Proteom in Zellkulturen, Organen und ganzen Organismen zu untersuchen.*

*Der Physiker **Prof. Thoralf Niendorf** ist auf einen Lehrstuhl für Experimentelle Ultrahochfeld-Magnetresonanz-Tomographie (MRT) an das Experimental and Clinical Research Center (ECRC) des MDC und der Charité berufen worden. Der Spezialist für bildgebende Verfahren von der Rheinisch-Westfälischen Technischen Hochschule (RWTH) Aachen hat im August 2009 die Leitung der MR-Anlage des ECRC auf dem Campus Berlin-Buch übernommen. Die Forschungsanlage mit einem der weltweit stärksten Magnetresonanz-Tomographen, einem 7-Tesla Ganzkörper-MRT, war im Januar 2009 von Bundesforschungsministerin Annette Schavan eingeweiht worden.*

*Der Neurowissenschaftler **Dr. James Poulet** ist an das Berliner Exzellenzcluster „NeuroCure" berufen worden und hat dort in Berlin-Mitte Anfang Juli 2009 seine Arbeit aufgenommen. Dr. Poulet, der über das MDC finanziert wird, erforscht wie neuronale Regelkreise Verhalten kontrollieren. Er war bis Sommer 2009 Postdoktorand an der École Polytechnique Fédérale de Lausanne, Schweiz, wo es ihm als erstem gelang, die intrazellluläre Aktivität zweier Nervenzellen der Großhirnrinde im wachen Tier aufzuzeichnen.*

*Im Rahmen der Initiative Systembiologie der Helmholtz-Gemeinschaft wechselte **Dr. Jana Wolf**, Humboldt-Universität zu Berlin, als Nachwuchsgruppenleiterin an das MDC. Die Nachwuchsgruppe wird zusätzlich über die BMBF Ausschreibung „Forschungseinheiten der Systembiologie-FORSYS" finanziert. Ihre Arbeit konzentriert sich auf die mathematische Modellierung zellulärer Prozesse.*

Awards
Preise

2008

Martin Bergmann
Wilhelm P. Winterstein-Preis, Deutsche Herzstiftung e. V.

Gudrun Erzgräber
Bundesverdienstkreuz, Bundesrepublik Deutschland

Martin Janz und Stephan Mathas
Curt-Meyer-Gedächtnispreis, Berliner Krebsgesellschaft e. V.

Friedrich Luft
Franz-Volhard-Medaille, Deutsche Gesellschaft für Nephrologie
Robert Tigerstedt Award, International Society of Hypertension
Lehrbär, Reformstudiengang Medizin der Charite – Universitätsmedizin Berlin

Nikolaus Rajewsky
1. Forschungspreis, Deutsche Gesellschaft für Gentherapie e. V.

Jan-Erik Siemens
Sofja Kovalevskaja-Preis, Alexander von Humboldt-Stiftung

Erich Wanker, Ulrich Stelzl, Christian Hänig, Gautam Chaurasia, Matthias Futschik
Erwin-Schrödinger-Preis, Hermann-von-Helmholtz-Gemeinschaft Deutscher Forschungszentren e. V.

2009

Norbert Hübner
Wissenschaftspreis für „Medizinische Grundlagenforschung", GlaxoSmithKline Stiftung

Friedrich Luft
Ross McIntyre Award, University of Nebraska

Raluca Niesner
Rahel-Hirsch-Stipendium, Charité – Universitätsmedizin Berlin

Klaus Rajewsky
Max-Delbrück-Medaille

Jens Reich
Carl-Friedrich-von-Weizsäcker-Preis, Nationale Akademie der Wissenschaften Leopoldina und Stifterverband für die Deutsche Wissenschaft

Francesca Spagnoli
Grant: European Research Council (ERC)

Thomas Willnow
Ehrendoktorwürde, Universität Aarhus, Dänemark

(Photo: E. Fesseler/Copyright: Helmholtz-Gemeinschaft Deutscher Forschungszentren)

Recipients of the Erwin Schrödinger Prize 2008
Prof. Erich Wanker (MDC), Dr. Ulrich Stelzl (MPI for Molecular Genetics), Prof. Jürgen Mlynek (President of the Helmholtz Association), Dipl.-Ing. Christian Hänig (MDC), Gautam Chaurasia, M.Sc., and Dr. Matthias Futschik (both HUB)(from left to right)

Preisträger des Erwin Schrödinger-Preis 2008
Prof. Erich Wanker (MDC), Dr. Ulrich Stelzl (MPI für molekulare Genetik), Prof. Jürgen Mlynek (Präsident der Helmholtz-Gemeinschaft), Dipl.-Ing. Christian Hänig (MDC), M.Sc. Gautam Chaurasia und Dr. Matthias Futschik (beide HUB)(v.l.)

Delbrück Fellows

Delbrück-Stipendium

Delbrück Fellowships at the MDC are intended to allow promising young scientists to carry out their own independent research. Delbrück Fellows have demonstrated that they are capable of conducting high quality research.

Fellows are associated with MDC host research groups and, therefore, receive lab space, infrastructure, and a research budget. The host research group guarantees the Fellow's independence in terms of research topic. In addition to MDC support, Fellows are expected to apply for external funding sources. Fellowships are typically granted for between three and 5 years.

Eligible are post-doctoral scientists with a strong recommendation from an MDC group leader. Applications are received by the MDC Scientific Director and reviewed by a selection committee. During the report period, the MDC supported 6 Delbrück Fellows.

Delbrück-Stipendien sind am MDC eingerichtet, um die frühe Unabhängigkeit junger, erfolgsversprechender Wissenschaftler zu ermöglichen. Sie sind für Wissenschaftler vorgesehen, die bereits nachgewiesen haben, dass sie hervorragende eigenständige, wissenschaftliche Arbeit leisten.

Die Delbrück-Stipendiaten sollen an bestehende Arbeitsgruppen des MDC Berlin-Buch angegliedert werden, in diesem Rahmen wird ihnen Raum und Infrastruktur sowie ein Sachmittelbudget zur Verfügung gestellt. Die gastgebende Forschungsgruppe garantiert Stipendiaten/Stipendiatin thematische Unabhängigkeit, die selbstständige Einwerbung von Drittmitteln wird erwartet. Das Stipendium wird in der Regel für eine Laufzeit von drei bis fünf Jahren gewährt.

Bewerben können sich Postdoktoranden auf Empfehlung eines Forschungsgruppenleiters aus dem MDC Berlin-Buch beim MDC-Vorstand. Es folgt dann eine Begutachtung in den MDC Gremien. Im Berichtszeitraum hat das MDC insgesamt 6 Stipendiaten gefördert.

International PhD Program

Internationales PhD-Programm

The MDC considers the training of new generations of researchers as a basic prerequisite for sustainable development and international scientific success. In 2003, an International PhD Program in "Molecular Cell Biology" was successfully established by the MDC and the Humboldt University (HU). Since that time, the MDC's offerings to graduate students have significantly expanded through the establishment of several new programs and rising numbers of applicants.

In 2007 the MDC and its partners – the HU, the Freie University (FU), and the FMP – were awarded a 6-year grant (3,6 Mio g) to establish the Helmholtz Graduate School in Molecular Cell Biology (HGS-MCB). This Graduate School offers a unified interdisciplinary platform for structured PhD training at the MDC and the consolidation of various graduate activities, such as training, supervision, measures to assist international students, quality control, and the creation of an alumni network. Training in research skills is further complemented by a structured Soft Skills Development Programme, ensuring that PhD students receive a well-rounded education that is essential for success in the modern scientific world. An annual review of the progress of all PhD students is carried out by PhD Project Committees that consist of 3 - 4 research group leaders from different research areas. Quality control and recording of all training activities will be carried out using the system of credits, analogous to the European Credit Transfer System, and students' achievements are summarized in the MDC PhD Certificate.

In addition, close collaboration between the MDC and the FU led to the establishment of the Helmholtz Research School in Molecular Neurobiology ('MolNeuro') in 2007, funded with 1.8 Mio g over 6 years. The training curriculum focuses on basic and advanced concepts of Molecular Neurobiology combined with a Soft Skill Development Programme that is organized by the Helmholtz Association for doctoral students from the MDC and other Helmholtz Research Schools. In addition the students organize an annual student conference called the Berlin Brain Days.

Die Ausbildung junger Nachwuchswissenschaftler ist für die zukünftige Entwicklung des MDC und seinen internationalen wissenschaftlichen Erfolg unverzichtbar. Aus diesem Grund richteten 2003 das MDC und die Humboldt-Universität zu Berlin (HU) das Internationale PhD-Programm "Molekulare Zellbiologie" ein, das sich in den folgenden Jahren erfolgreich etablieren konnte.

Seitdem hat sich das Angebot für die Doktoranden durch den Aufbau neuer Doktorandenprogramme erweitert: 2007 erhielten das MDC zusammen mit seinen Partnern-HU, Freie Universität Berlin (FU) sowie das Leibnitz Institut für Molekulare Pharmakologie (FMP) die Zusage die Helmholtz Graduate School „Molecular Cell Biology" zu gründen. Sie wird aus dem Impuls- und Vernetzungsfonds des Präsidenten der Helmholtz-Gemeinschaft mit insgesamt 3,6 Mio Euro über 6 Jahre gefördert und ist die Grundlage für eine interdisziplinäre, strukturierte Ausbildung aller Doktoranden am MDC. Das Ausbildungs-Curriculum beinhaltet neben dem gesamten Spektrum der molekularen Zellbiologie auch die Entwicklung zusätzlicher Schlüsselqualifikationen, sogenannter „Soft Skills". Es stellt sicher, dass die Doktoranden eine vielseitige und umfassende Ausbildung erhalten, die für den Erfolg in der modernen Wissenschaftswelt entscheidend ist.

Die jährliche Leistungsbewertung aller Doktoranden wird von PhD-Projektausschüssen durchgeführt, die sich aus drei bis vier Forschungsgruppenleitern aus verschiedenen Forschungsbereichen zusammensetzen. Die Qualitätskontrolle und die Dokumentation aller Ausbildungsmaßnahmen werden entsprechend dem europäischen Leistungspunktsystem ECTS (European Credit Transfer System) durchgeführt. Nach Abschluss ihrer Promotion erhalten die Absolventen ein MDC-Promotionszertifikat mit ihren Leistungen und können in das Alumni-Netzwerk aufgenommen werden.

Die enge Zusammenarbeit zwischen MDC und FU führte 2007 zur Gründung der „Helmholtz Research School Molecular Neurobiology (HRS „MolNeuro"), die mit 1,8 Millionen Euro für sechs Jahre gefördert wird. Das Programm bietet eine fachspezifische Ausbildung im Bereich der molekularen Neurobiologie, die durch zentrale Helmholtz-Soft Skill Kurse, die mit Doktoranden

Photo: David Ausserhofer / Copyright: MDC

Yinth Andrea Bernal-Sierra, a PhD student in the Research School Molecular Neurobiology (Research group: Prof. Gary Lewin), examines cultured neurons with a fluorescence microscope.
Yinth Andrea Bernal-Sierra, eine Doktorantin der Research School Molecular Neurobiology (Forschungsgruppe: Prof. Gary Lewin), untersucht kultivierte Neuronen mit einem Fluoreszenzmikroskop.

The Helmholtz Research School in Translational Cardiovascular and Metabolic Medicine (TransCard) has been funded for 2009-2015. It fosters translational research with targeted education of graduate students, scholarships, travel grants, and IT-infrastructure. PhD students in the Cardiovascular and Metabolic Disease Program are affiliated with both basic science and clinical research groups and actively develop the program by organizing seminars, retreats, and conferences. The Program offers lectures, summer schools, and e-learning and encourages interactions not only between students and faculty in Berlin, but also with our international partner universities.

In 2009 the Berlin Institute Medical Systems Biology and the Center for Genomics and Systems Biology of the New York University started their joint PhD-Exchange- Programme. The students are working on collaborative projects with a scientific focus on post-transcriptional gene regulation in the elucidation of

anderer „Helmholtz Research Schools" veranstaltet werden, ergänzt wird. Jährlich organisieren die Doktoranden mit anderen Doktorandenprogrammen aus dem Neurobiologie Bereich in Berlin die Berlin Brain Days.

Im Januar 2009 hat ein weiteres Helmholtz-Kolleg, die "Helmholtz Research School in Translational Cardiovascular and Metabolic Medicine" (TransCard), seine Arbeit aufgenommen. Es wird für die nächsten 6 Jahre mit insgesamt 1,8 Millionen Euro durch den Impuls und Vernetzungsfonds der Helmholtz-Gemeinschaft gefördert. Das Programm bietet eine fachspezifische Ausbildung durch Vorlesungen, Summer Schools und E-Learning im Herz-Kreislauf-Bereich sowie der Metabolischen Medizin, die durch zentrale Helmholtz-Soft Skill Kurse ergänzt werden.

Ebenfalls 2009 startete das gemeinsame Doktorandenprogramm des Berlin Institute Medical Systems Biology (BIMSB) und dem Zentrum für Genomics und Systembiologie der New York Universität. Doktoranden dieses Programms bearbeiten Kooperationsprojekte zwischen Wissenschaftlern des MDCs sowie der Universität New York. Alle Doktoranden arbeiten sowohl in Berlin als auch in New York.

biological systems. All the participating students split their time between Berlin and New York.

Currently, approximately one quarter of all MDC PhD students are selected through a vigorous international recruitment process. Around 180 PhD students are working towards their PhD degree at the MDC and 70 PhD students visit the MDC per year to work on collaborative projects. An annual scientific symposium (PhD Retreat) is organized by the PhD students of the MDC and FMP to promote further networking between the students.

During the 2009 PhD student selection process, 779 applications from 69 countries were reviewed. Of those applicants, 23 PhD students were accepted into the different Programs. The MDC also has a Fellowship Committee that awards scholarships to outstanding candidates who develop and present a research project.

The degree of Dr. rer. nat. (German equivalent of a PhD degree) is either awarded by the partner universities or may be obtained through the student home university. Eligible are graduate students of the life sciences holding a degree comparable to the German Diploma or a MSc with a research thesis is typically required for applicants.

Am MDC arbeiten ständig rund 180 Doktorandinnen und Doktoranden. Etwa weitere 70 im Jahr besuchen das MDC, um an Partnerprojekten mitzuarbeiten. Die Doktoranden des MDC und des FMP organisieren jährlich ein wissenschaftliches Symposium (Doktoranden-Klausurtagung) in Brandenburg sowie einen Wettbewerb für den besten Vortrag, das beste Poster auf dem Campus.

Zurzeit wird etwa ein Viertel aller MDC-Doktoranden in einem strengen internationalen Bewerbungsverfahren ausgewählt. Während des Auswahlverfahrens 2009 bewarben sich 779 Kandidaten aus 69 Ländern. Aus der Gruppe der Bewerber wurden insgesamt 23 Doktorandinnen und Doktoranden in die unterschiedlichen Programme aufgenommen. Zusätzlich vergibt der Stipendienausschuss am MDC in jedem Jahr Stipendien, für die sich Doktoranden und Post-Doktoranden mit herausragenden Forschungsprojekten bewerben können. Zur Bewerbung berechtigt sind Absolventen der Lebenswissenschaften mit einem deutschen Diplom oder ein Masterabschluss (Master of Science - MSc) einschließlich einer MSc-Forschungsarbeit. Den Doktoranden wird der Titel Dr. rer. nat. (die deutsche Entsprechung zum PhD) von einer der Partneruniversitäten verliehen. Ebenso können die Studierenden an einer ihrer Heimatuniversität promovieren.

Congresses and Scientific Meetings

Kongresse und Wissenschaftliche Tagungen

2008

11 January	**Max Delbrück Lecture , Prof. Michael Botchan, University of California, Berkeley, USA** Organizer: Prof. M. Gossen, MDC
18 January	**Neujahrsempfang des Campus Berlin-Buch** Organizer: MDC/BBB GmbH
08 February	**Max Delbrück Lecture, Prof. Ruth Lehmann, Developmental Genetics Program, Skirball Institute and Howard Hughes Medical Institute New York, University School of Medicine, New York, USA** Organizer: Prof. N. Rajewsky, MDC
16 February	**HNO-Symposium des Helios Klinikums** Organizer: Helios–Klinikum Berlin-Buch
26-29 March	**International Conference "Invasion and Metatasis"** Organizer: Prof. W. Birchmeier, MDC
24-25 April	**Laborbaukonferenz** Organizer: R. Streckwall, MDC
14-17 May	**International Conference „Development and Function of Somatosensation and Pain"** Organizer: Prof. G. Lewin, MDC
26-28 May	**International Symposium "Adoptive T Cell Therapy"** Organizer: Prof. W. Uckert, MDC
07 June	**13. Bucher Nephrologisches Symposium** Organizer: Prof. F. Luft, MDC/FVK
12-14 June	**International Berlin Summer Meeting "Computational & Experimental Molecular Biology"** Organizer: Prof. N. Rajewsky, MDC
14 June	**Lange Nacht der Wissenschaften** Organizer: Campus Berlin-Buch
17 June	**Max Delbrück Lecture, Prof. K. Chien, MGH Cardiovascular Research Center, Massachusetts General Hospital, Richard B. Simches Research Center, Boston, USA** Organizer: Dr. D. Besser, MDC
19 - 21 June	**International Conference "Transposition and Animal Biotechnology"** Organizer: Dr. Zs. Izsvak, MDC
27 June	**Festveranstaltung zu Ehren der Verabschiedung von Dr. Gudrun Erzgräber, Geschäftsführerin der BBB GmbH und Verleihung des Bundesverdienstkreuzes** Organizer: Campus Berlin-Buch
07 -11 July	**Basis Gene Mapping /Linkage Analysis Course** Organizer: Forschungsgruppe Bioinformatik, MDC mit Dr. M. Nothnagel, Uni Kiel
11 July	**Max Delbrück Lecture, Dr. Helen H. Hobbs HHMI, UT Southwestern Medical Center Dallas, USA** Organizer: Dr. Daniel Besser, MDC
08-10 October	**15th Annual Meeting of the German Society of Gene Therapy, DG-GT e.V. & 6th Annual Workshop "Viral Vectors and Gene Therapy" of the Gesellschaft für Virologie, GfV** Organizer: Prof. W. Uckert, MDC mit Charité und HU Berlin
29 October	**Symposium: Junior Group Leader Position Systems Biology am MDC** Organizer: Prof. N. Rajewsky, MDC
29-30 October	**NMR-Symposium: "Challenging Biological Systems Systems by NMR Spectroscopy"** Organizer: Prof. H. Oschkinat, FMP
07 November	**Max Delbrück Lecture, Prof. Dr. Jonathan Weissman Howard Hughes Medical Institute San Francisco, USA** Organizer: Prof. Th. Sommer, MDC

07-08 November	**7. Bucher Hämatologieforum** Organizer: Beckman Coulter GmbH + Prof. W.-D. Ludwig, RRK
12-14 November	**International Symposium on Chemical Biochemistry** Organizer: Prof. Dr. H. Oschkinat, FMP
01-02 December	**"Capital Market Day 2008" Fa. Fresenius SE,** Organizer: Bad Homburg
04-05 December	**"Brain Tumor Meeting"** Organizer: Helmut Kettenmann, MDC mit Helios Klinikum Berlin und Charité Campus, Virchow-Klinikum
08 December	**Information workshop "EMBO: promoting excellence in the molecular life sciences in Europe" Speaker: Dr Jan Taplick, EMBO Deputy Executive Director, EMBO Fellowship Programme Manager and Information workshop 'Career Pathways'** Organizer: PhD Program at the MDC, Dr. O. Seumenicht and Dr. J. Droese
10-12 December	**Berlin Brain Days 2008 veranstaltet von sieben, in Berlin angesiedelten, neurowissenschaftlichen Promovierendenprogrammen**

2009

20 January	**Neujahrsempfang des Campus Berlin-Buch** Organizer: MDC/BBB GmbH
11 November - 15 March	**Ausstellung mit Zeichnungen von Berliner Schülern anlässlich des 200. Geburtstages von Darwin** Organizer: Gläsernes Labor der BBB GmbH
21 April - 30 June	**Ausstellung "Frauen in Naturwissenschaft und Technik" MDC Frauenbeauftragte** Organizer: Dr. H. Haase
23-24 April	**Laborbaukonferenz R. Streckwall** Organizer: MDC
12 June	**Wissenschaftshistorisches Kolloquium anläßlich des 80. Geburtstages von Prof. Dr. em. Heinz Bielka und des 70. Geburtstages von Prof. Dr. em. Jens Reich** Organizer: MDC
13 June	**Lange Nacht der Wissenschaften** Organizer: Campus Berlin-Buch
19 June	**Max Delbrück Lecture, Dr. Neal Copeland, Institute of Molecular and Cell Biology, Singapore** Organizers: Carmen Birchmeier and Walter Birchmeier
20 June	**14. Bucher Nephrologisches Symposium** Organizer: Prof. F. Luft, FVK
03 July	**NEW MDC SCIENTISTS present their work Max Delbrück Center for Molecular Medicine (MDC),** Organizer: Berlin Institute for Medical Systems Biology (BIMSB)
10 July	**Max Delbrück Lecture , Dr. Stephen Cohen, Temasek Life Sciences Laboratory, Singapore** Organizer: Prof. Th. Sommer, MDC
22-26 September	**International EMBO Conference Series "Ubiquitin and Ubiquitin-like Modifiers in Health and Disease"** Organizer: Prof. T. Sommer, MDC
08-10 October	**International Berlin Summer Meeting "Computational & Experimental Molecular Biology"** Organizer: Prof. N. Rajewsky, MDC
09-13 November	**Basis Gene Mapping /Linkage Analysis Course Forschungsgruppe Bioinformatik** Organizer: MDC mit Dr. M. Nothnagel, Uni Kiel
2009 December	**Berlin Brain Days 2009 veranstaltet von sieben, in Berlin angesiedelten, neurowissenschaftlichen Promovierendenprogrammen.**

Seminars

Seminare

2008

Speaker		Institute	Title
Thomas	Bayer	Department of Psychiatry, University Medical Center, Göttingen, Germany	Neurodegeneration in Alzheimer's disease: novel findings from mouse models
Thomas	Biederer	Yale University, New Haven , USA	SynCAM adhesion molecules: Working hand-in-hand to organize synapses
Gerard	Borst	Erasmus University Rotterdam, Rotterdam, Netherlands	Development of the calyx of Held synapse
Grzegorz	Bulaj	University of Utah, USA	Discovery and Development of Venom-Derived Sodium Channel Blockers as Therapeutics for Pain
Federico	Calegari	CRTD, Center for Regenerative Therapies, Dresden, Germany	Role of cell cycle length in mammalien neurogenesis
David	Callen	The University of Adelaide Australia, Adelaide Australia	Chromosome16 Breast Cancer Tumor Suppressors
Wei	Chen	Max Planck Institute for Molecular Medicine, Berlin, Germany	Application of Illumina/Solexa sequencing technology in genetic research
Cyrille J.	Cohen	Bar-Ilan University, Ramat Gan, Israel	Engineering the T-lymphocyte immune response against cancer
Jürgen	Cox	Max Planck Institut für Biochemie, Martinsried, Germany	Mass spectrometry-based proteomics with high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide quantification
Karin	de Visser	Dept. Molecular Biology, The Netherlands Cancer Institute, Amsterdam, Netherlands	The inflammatory tumor microenvironment and its impact on cancer development
Ivan	Dikic	Goethe University Frankfurt/Main, Frankfurt, Germany	Targeting Ubiquitin networks
Valentin	Djonov	Department of Medicine Gross Anatomy and Vascular Biology, University of Fribourg, Switzerland	Novel insights into intussusceptive angiogenesis
Andreas	Ettinger	MPI für Molekulare Zellbiologie und Genetik, Dresden, Germany	Trash or Treasure? – the Midbody in Differentiation and Cancer
Reinhard	Fässler	Max Planck Institute of Biochemistry	MartinsriedGermanyGenetic analysis of integrin signaling in mice
Jonathan	Flint	Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom	The genetic architecture of complex traits in the mouse
Jean-Marc	Fritschy	University of Zurich, Zurich, Switzerland	GABAA receptors and homeostatic synaptic plasticity
Armin	Giese	LMU Munich, Munich, Germany	Prions and Parkinson: From single particle analysis of protein aggregation to new therapeutics

Speaker		Institute	Title
Magdalena	Götz	GSF – National Research Center for Environment and Health, Neuherberg, Germany	Neurogenesis from glial cells: new views on reactive gliosis and repair
Francois	Guillemot	Nat. Institut for Medical Research, London, United Kingdom	Transcriptional control of neurogenesis and neuronal migration in the mouse brain
Jana	Hartmann	TU Munich, Munich, Germany	mGluR-dependent signaling in cerebellar Purkinje cells
Ronald	Hay	University of Dundee, England	How SUMO targets proteins for proteasomal degradation
Matthias W.	Hentze	EMBL, Germany	Understanding metabolic regulatory networks: iron metabolism and its diseases
Eric	Honoré	Institut de Pharmacologie, Moléculaire et Cellulaire, Physiopathologie Cardiovasculaire CNRS, Valbonne, France	Sensing pressure with K2P channels
Sebastian	Jessberger	Institute of Cell Biology, ETH Zurich, Zurich, Switzerland	Fate plasticity of adult neural stem cells and molecular mechanisms of neuronal maturation
Sven-Eric	Jordt	Yale University School of Medicine, New Haven, USA	TRP channels in thermosensation and respiratory reflex control
Oktay	Kirak	Whitehead Institut for Biomedical Research, Cambridge, United Kingdom	When T Cells Meet Eggs
Oleg	Krishtal	Bogomoletz Institute Kiev, Ukraine	Enigma of P2X3 receptors: how to reconcile the properties with putative function?
Thomas	Kuner	Institut für Anatomie und Zellbiologie Uni Heidelberg, Germany	Synaptic Inhibition Accelerates Odor Discrimination in Mice
Markus	Landthaler,	The Rockefeller University, New York, USA	Decoding posttranscriptional regulatory networks
Peter	Laslo	Leeds Institute for Molecular Medicine (University of Leeds), Leeds, United Kingdom	Gene regulatory networks that regulate macrophage development
Catherine	Lubetzki	Hôpital de la Salpêtrière Paris, France	Success or failure of myelin repair
Marcy	MacDonald	Massachusetts General Hospital, USA	Genetics-directed approach to Huntington's disease therapeutics
Harvey	MacMahon	MRC Laboratory of Molecular Biology, Cambridge, United Kingdom	Sculpting Cell Membranes: Understanding pathways of endocytosis and exocytosis
Marco	Mank	MPI, Martinsried, Germany	Improved Genetically-Encoded Calcium Indicators Based on Troponin C
Peter	McNaughton	University of Cambridge, United Kingdom	Why pain gets worse – role of the pacemaker current Ih in nociception
Edwin	Monuki	University of California, Irvine, USA	The Morphogen and Selector Gene Network in the Dorsal Telencephalon

Speaker		Institute	Title
Kiyoshi	Mori	Kyoto University Graduate School of Medicine, Department of Medicine and Clinical Science, Kyoto, Japan	Biology of a siderophore-binding protein – A common molecular mechanism underlying mesenchymal-epithelial transition, kidney protection, innate immunity and anemia
Richard I.	Morimoto	Northwestern University, USA	Regulating Proteostasis in Aging and Neurodegenerative Disease
Tobias	Moser	University of Goettingen, Goettingen, Germany	Molecular physiology of the hair cell ribbon synapse
Pierluigi	Nicotera	University of Leicester, Leicester, United Kingdom	Subcellular programmes for neurodegeneration
Stephen	Nutt	WEHI Institute of Medcial Research, Australia	The transcriptional regulation of lymphocyte differentiation
Raz	Palty	Department of Physiology, Zlotowski Center for Neuroscience, Beer-Sheva, Israel	NCLX is an essential component of mitochondrial Na+/Ca2+ exchange
Leena	Peltonen	National Public Health Institute Helsinki, Finland	Genes behind rare and common neurological diseases: Lessons from a genetic isolate
Alexandre	Prat	Université de Montréal, Montreal, Canada	Leukocyte adhesion to and trafficking across brain ECs
Bernd	Pulverer	United Kingdom	Scientific Publishing
Maike	Sander	University of California, California, United States	Temporal Control of Progenitor Cell Pluripotency in the Pancreas
Martin	Schmelz	Universität Heidelberg, Heidelberg, Germany	Modulation of axonal excitability and pain – single fiber recordings in human and pig
Ralf	Schneggen-burger	Swiss Institute of Technology, Switzerland	Calcium regulation of transmitter release at a larger CNS synapse
Gunnar	Schütz	Bayer Schering Pharma AG, Germany	Molecular MRI: Magnetic Resonance Animal Imaging in Basic Research
Barbara	Seliger	Marthin Luther Universität Halle, Halle ,Germany	Many ways lead to MHC class I abnormalities
Stephan	Sigrist	University of Wuerzburg, Germany	Shedding light on synapse assembly and plasticity
Peter	Sonderegger	University of Zurich, Zurich, Switzerland	Local proteolysis at CNS synapses – a key for cognitive functions ?
Theresia	Stradal	Signaling and Motility Group Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany	Regulation of different actin based protrusions at the cell periphery
Ursula	Strobl	German Research Center for Environmental Health, Helmholtz Zentrum München, Neuherberg, Germany	The Epstein-Barr viral proteins LMP1 and EBNA2 and their cellular homologues CD40 and Notch in B cell proliferation and differentiation

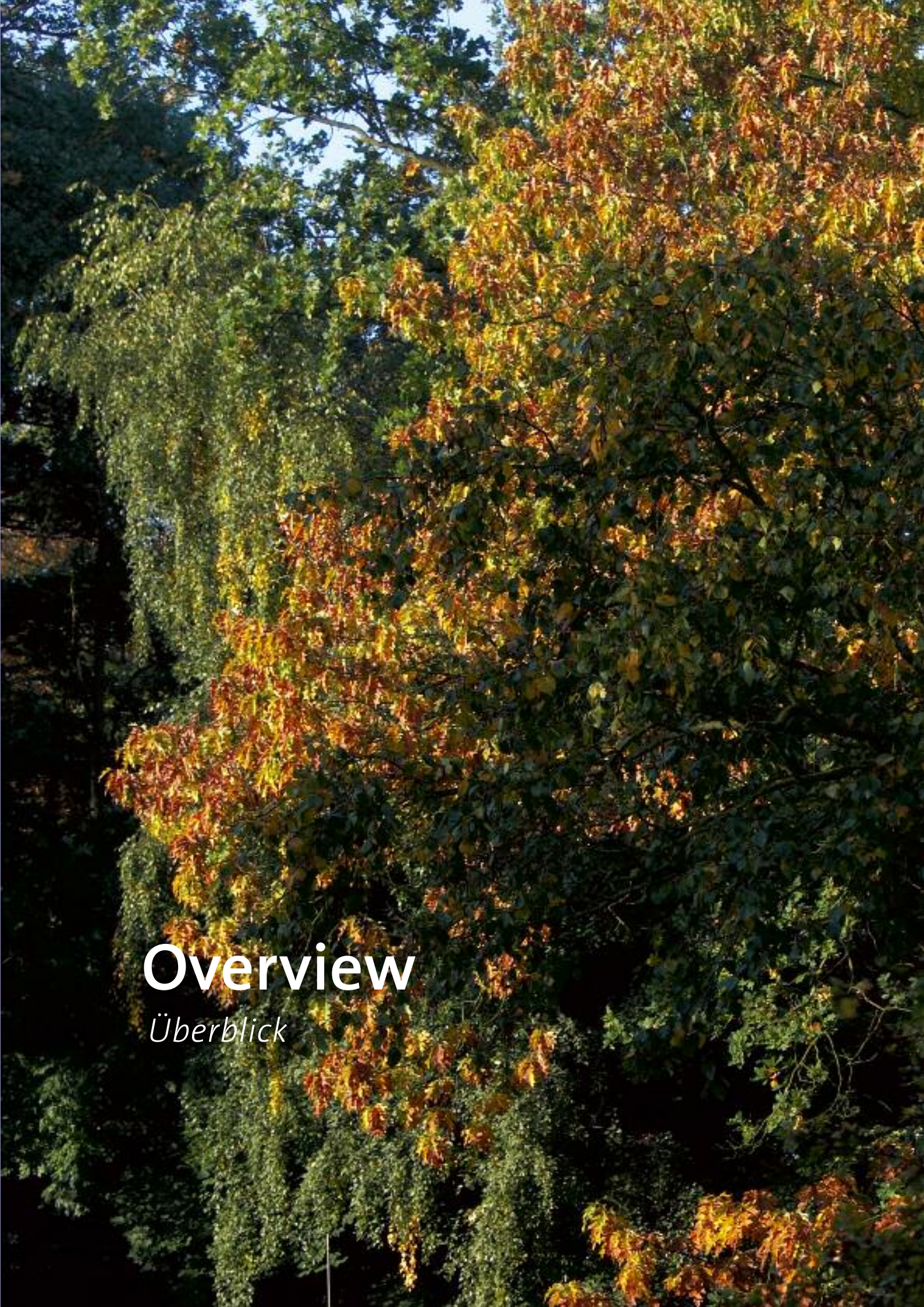
Speaker		Institute	Title
Albrecht	Stroh	Technical University/ Neurosciences, Munich, Germany	Modulation of embryonic stem cell differentiation with automated temporally precise optogenetic stimulation
Haruo	Sugi	University of Tokyo, Tokyo, Japan	Electron microscopic demonstration of cross-bridge recovery stroke in living muscle thick filaments using the gas environmental chamber
Holger	Taschen-berger	Max Planck Institute for Biophysical Chemistry, Goettingen, Germany	Developmental plasticity at the calyx of Held synapse
Andrei	Thomas-Tikhonenko	Prof. of Pathology at the University of Pennsylvania, USA	microRNAs and oncogenic signaling: searching for miRaculous cancer cures
Matthias	Treier	EMBL, Heidelberg, Germany	A Sall protein complex mediates pluripotency
Bahar	Tunctan	Mersin University Turkey, Turkey	Interactions between cytochrome P450 4A, cycloxygenase and nitric oxide synthase during endotoxemia: therapeutic implications for inflammatory diseases
David	Van Vactor	Harvard Medical School, Boston, USA	Tyrosine Kinase and Phosphatase Regulation of Synapse Development in Drosophila
David	Van Vactor	Harvard Medical School, Boston, USA	Tyrosine Kinase and Phosphatase Regulation of Synapse Development in Drosophila
Devadasan	Velmurugan	University of Madras, Center of Adv. Studies in Crystallography and Biophysics, India	Contributions to Macromolecular Phasing, Structure Analysis and Modelling
Richard	Warth	Universität Regensburg, Germany	Task K+ Channels: adrenal gland development and function
Deborah J.	Watson	University of Pennsylvania, Department of Neurosurgery, Philadelphia, USA	Normal and pathotropic migration of SVZ neural progenitors in rodent, canine and feline models
Allan	Weissman	NCI Frederick, Maryland, USA	RING Finger Ubiquitin Ligases in ER-Associated Degradation and as Targets in Cancer
Marius	Wernig	Whitehead Institute for Biomedical Research, Cambridge, United Kingdom	Stem Cells and Epigenetic Reprogramming
Klaus	Willecke	University of Bonn, Bonn, Germany	Expression and function of connexins – studies with targeted mouse mutants
Jochen	Wittbrodt	EMBL, Heidelberg, Germany	Seeing is believing: Novel insights into vertebrate eye development
Jana	Wolf	Institute of Biology, Humboldt University Berlin, Germany	Mathematical modelling of signal transduction for the analysis of oncogenic mutations

2009

Speaker		Institute	Title
David G.	Attwell	University College London, London, Germany	Neurotransmitter signaling to oligodendrocyte lineage cells: its role in CNS development and neurological disorder
Joao	Barata	Cancer Biology Unit, Instituto de Medicina Molecular, Lisbon, Portugal	PTEN inactivation in primary T cell acute leukemia: More than the canons
Heinrich	Betz	MPI für Hirnforschung, Frankfurt, Germany	How to make inhibitory synapses
Knut	Biber	University Medical Center Groningen, Groningen, Netherlands	IL-6-type cytokines and adenosine receptors in neuroprotection
Thomas	Brand	Theodor-Boveri-Institut für Biowissenschaften Biozentrum, Universität Würzburg, Germany	Molecular signals that control induction and left- right asymmetry of proepicardium formation in the chick embryo
Michael	Brecht	Bernstein Center, Berlin, Germany	Cellular mechanisms underlying hippocampal activity in freely moving rodents
Joel	Buxbaum	The Scripps Research Institute, La Jolla, CA, USA	Can amyloid precursors chaperone each other? Transthyretin and neurodegenerative diseases
Michael	Dodt	TU Dresden / MPI for Cell Biology and Genetics Dresden, Germany	Generation and evaluation of impulse-media for the computer aided visual therapy
David L.	Feldman	Novartis Institutes for Biomedical Research Inc. E. Hanover, NJ, USA	Renin inhibitor treatment – new insights
Vincent	Fleury	Laboratoire Matière et Systèmes Complexes Paris, France	Hydrodynamic effects in animal morphogenesis: does evolution follow a streamline?
Gilles	Fortin	Institut de Neurobiologie Alfred Fessard, Gif sur Yvette, France	Central control of breathing: views from the embryo
Yasujuki	Fujita	MRC, University of London, London, United Kingdom	Charaterization of the interface between normal and transformed epithelial cells
Tony	Green	Head, University of Cambridge Department of Haematology, Cambridge Institute for Medical Research, Cambridge, United Kingdom	Molecular pathogenesis of the myeloproliferative disorders
Robert	Grosse	Universität Heidelberg, Germany	Formin-controlled mechanisms of cell morphology and invasion
Manuel	Guzman	Complutense University, Madrid, Spain	Mechanisms of cannabinoid-induced cancer cell death
Uwe-Karsten	Hanisch	Georg-August-Universität, Göttingen, Germany	Microglial responses to endogenous TLR agonists
Eric B.	Haura	The H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA	Mechanisms and biomarkers of tyrosine kinase inhibitors identified through phospho proteomics
Andreas	Herrlich	Whitehead Institute for Biomedical Research, Cambridge, USA	A shRNA-based approach to identify novel kinases and phosphatases that regulate EGF ligand cleavage

Speaker		Institute	Title
Sergey	Kasparov	Department of Physiology and Pharmacology, University of Bristol, United Kingdom	Use of cell-specific viral gene expression in neuroscience
Frank	Kirchhoff	MPI für Experimentelle Medizin, Göttingen, Germany	Interactions of neurons and glia in the central nervous system – transgenic mice and two-photon imaging
André	Kléber	Institute of Physiology, University of Bern, Switzerland	Role of Cx43 in ventricular myocardium
Kaiqin	Lao	Applied Biosystems, Foster City, USA	mRNA Seq: Whole-transcriptome analysis of a single cell
Uwe	Maskos	Institut Pasteur, Paris, France	Dissecting the nicotinic control over the dopamine system in vivo
Dies	Meijer	Erasmus University, Rotterdam, Netherlands	Claw Paw meets the Adams family: A novel signaling axis in peripheral nerve development
Alex	Meissner	Harvard Stem Cell Institute, Cambridge, USA	Epigenomics and Pluripotency
Slavoljub	Milosevic	Institut of Molecular Immunology, München, Germany	Identification of antigens, epitopes and T-cell receptors for vaccine development and adoptive immunotherapy
Gerhard	Moldenhauer	Deutsches Krebsforschungszentrum Heidelberg, Germany	Development of a L1-CAM (CD171)-specific anti-cancer antibody
Peter	Mombaerts	Max-Planck Institute of Biophysics	Frankfurt Germany Olfaction Targeted
Emma	Morris	University College London Hospital NHS Trust, London, Great Britain	TCR Gene Transfer: Novel Immunotherapy for Leukaemia
Manabu	Murakami	Akita University School of Medicine, Japan	Modified sympathetic nerve system activity with over-expression of the voltage-dependent calcium channel beta3 subunit
Uwe	Ohler	Institute for Genome Sciences and Policy, Duke University, Durham, NC, USA	High-throughput data generation and computational models for eukaryotic promoters
Shgeo	Okabe	Department of Cellular Neurobiology, University of Tokyo, Tokyo, Japan	Visualization of synapse formation and remodeling in excitatory
Michael	Olivier	Medical College of Wisconsin, USA	Why do we get fat? Integrated genomic and proteomic analysis of human obesity
Tobias	Pischon	German Institute of Human Nutrition (DIfE), Potsdam-Rehbrücke, Germany	Obesity, Adipokines and Cardiovascular Risk
Alexey	Ponomarenko	Abt. für Klinische Neurobiologie, Neurologische Universitätsklinik, Heidelberg, Germany	Generation of rhythms in the hippocampus: insights from genetic modifications of the network clocks
Richard	Ransohoff	Neuroinflammation Research Center, Cleveland Clinic, Cleveland, USA	Early cortical demyelination gives a view of MS pathogenesis from the outside-in

Speaker		Institute	Title
Juri	Rappsilber	Wellcome Trust Centre for Cell Biology, Edinburgh, Scotland	The protein composition of chromatin
Ingmar	Riedel-Kruse	California Institute of Technology, Pasadena, USA	Synchrony among sperms, neurons, and genes
Rafael	Roesler	Federal University of Rio Grande do Sul, Porto Alegre, Brazil	The gastrin – releasing peptide receptor as a therapeutic target in cancer and neurological disorders
David	Rubinsztein	Cambridge Institute for Medical Research, Cambridge, United Kingdom	Autophagy and Neurodegeneration
Pierre	Savatier	INSERM U846 Stem Cell and Brain Research Institute, Bron, France	Dissecting the mechanisms of STAT3 and Nanog-dependent self-renewal in mouse embryonic stem cells
Peter	Scheiffele	University of Basel, Basel, Switzerland	Molecular Mechanisms of Neural Circuit Assembly
Günther	Schütz	DKFZ, Heidelberg, Germany	The nuclear receptor Tailless is crucial for adult neurogenesis and brain tumor formation
Nicholas D.	Socci	Memorial Sloan Kettering Cancer Center, New York, USA	Deep sequencing of small RNA profiles in cancer
Uwe	Strähle	University of Heidelberg and Institute of Toxicology and Genetics Forschungszentrum Karlsruhe, Germany	Adult neurogenesis in the zebrafish
Shunichi	Takeda	Center for Frontier Medicine, Kyoto University, Japan	Reverse genetic study of DNA damage response by using the chicken DT40 cell line, and its application to chemical genetics and SILAC-based proteomic analysis
Dominic	Tölle	EBI / Cambridge University, Cambridge, United Kingdom	Meredys, a particle-based stochastic simulation software
Klaus	Unsicker	University of Heidelberg, Germany	Role of GDF-15, a novel member of the TGF-βs, in motoneuron maintenance and peripheral myelination
Pierre	Van der Bruggen	Ludwig Institute for Cancer research, Brussel, Switzerland	Is it possible to correct the anergy of human tumor-infiltrating lymphocytes?
Cinzia	Volontè	Institute of Neurobiology and Molecular Medicine , Rome, Italy	Once upon a time ..... P2 receptors, P1 receptors, ectonucleotidases and nucleosi de/nucleotide transporters got all together in a compartment at the plasma membrane ..... and the purinergic signaling began
Manfred S.	Weiss	EMBL Outstation, DESY, Hamburg, Germany	Structural analysis of enzyme complexes in biosynthetic pathways and Use of long X-ray wavelengths in macromolecular crystallography
Herbert	Zimmermann	University of Frankfurt, Frankfurt, Germany	Purinergic signaling in adult neurogenesis



Overview

Überblick

The Helmholtz Association

Die Helmholtz-Gemeinschaft

The Helmholtz Association

The Helmholtz Association (HGF) is the largest scientific organization in Germany with 28,000 employees in 16 research centers and an annual budget of around 2.1 billion Euros. The research centers of the Helmholtz Association are divided into six research sectors: Energy, Earth and Environment, Health, Key Technologies, Structure of Matter, and Transport and Space.

Eight centers have a focus on the field of Health research in six research programs: cancer, cardiovascular and metabolic diseases, functions and dysfunctions of the nervous system, infections and immunity, environmentally-linked health problems and the systemic analysis of multifactorial diseases. The most significant research activities are clustered at five Helmholtz Health centers: the *Deutschen Krebsforschungszentrum* (DKFZ) in Heidelberg, the *Helmholtz Zentrum München für Gesundheit und Umwelt* (HMGU), the *Helmholtz-Zentrum für Infektionsforschung* (HZI) in Braunschweig, and at the MDC as well as at the future *Deutschen Zentrum für Neurodegenerative Erkrankungen* (DZNE) in Bonn. Other important health-related work is underway at the following Helmholtz centers: the *Forschungszentrum Jülich* (FZJ), *Helmholtz-Zentrum für Schwerionenforschung* (GSI) in Darmstadt, the *Forschungszentrum Geesthacht* (GKSS) and the *Helmholtz-Zentrum für Umweltforschung* (UFZ) in Leipzig. The focus of these centers lies in carrying out excellent, dynamic basic research and translating what is learned to the clinic. The MDC participates in three thematic programs, and is the coordinator of the program in Cardiovascular and Metabolic Diseases.

Program-Oriented Funding

Since the establishment of the Helmholtz e.V. in the fall of 2001, research within the Helmholtz Association has been strategically restructured. The six research fields (see above) encompass different research programs that are conducted by one or more of the individual centers.

Central to the research reform is the Program-Oriented Funding (POF) mechanism. Funding now goes to scientific programs (on a competitive basis) rather than to the centers. Every program is evaluated by an international panel of experts. This evaluation is the

Die Helmholtz-Gemeinschaft

Die Helmholtz-Gemeinschaft ist mit ihren rund 28 000 Mitarbeiterinnen und Mitarbeitern in 16 Forschungszentren sowie einem Jahresbudget von rund 2,8 Milliarden Euro die größte Wissenschaftsorganisation Deutschlands.

Die Forschungszentren der Helmholtz-Gemeinschaft arbeiten in sechs Forschungsbereichen: Energie, Erde und Umwelt, Gesundheit, Schlüsseltechnologien, Struktur der Materie sowie Verkehr und Weltraum.

Im Forschungsbereich Gesundheit kooperieren 8 Helmholtz-Zentren in 6 Forschungsprogrammen: Krebsforschung, Herz-Kreislauf- und Stoffwechselerkrankungen, Funktion und Dysfunktion des Nervensystems, Infektion und Immunität, Umweltbedingte Störungen der Gesundheit sowie Systemische Analyse von multifaktoriellen Erkrankungen. Die wichtigsten Forschungsaktivitäten sind an 5 Helmholtz-Gesundheitszentren angesiedelt: am Deutschen Krebsforschungszentrum *(DKFZ) in Heidelberg, am* Helmholtz Zentrum München für Gesundheit und Umwelt *(HMGU), am* Helmholtz-Zentrum für Infektionsforschung *(HZI) in Braunschweig und am MDC sowie in Zukunft auch am* Deutschen Zentrum für Neurodegenerative Erkrankungen *(DZNE) in Bonn. Darüber hinaus leisten die Helmholtz-Zentren:* Forschungszentrum Jülich *(FZJ),* Helmholtz-Zentrum für Schwerionenforschung *(GSI) in Darmstadt,* Forschungszentrum Geesthacht *(GKSS) und* Helmholtz-Zentrum für Umweltforschung *(UFZ) in Leipzig, wichtige Beiträge. Der Fokus liegt auf einer starken, exzellenten und dynamischen Grundlagenforschung und der Übertragung der gewonnenen Erkenntnisse in die Klinik. Das MDC ist an drei Programmen beteiligt. Das Programm, Herz-Kreislauf- und Stoffwechselerkrankungen' wird federführend vom MDC getragen.*

Die Programmorientierte Förderung

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, die von einem oder mehreren Zentren getragen werden.

Ressourcen werden nicht mehr einzelnen Institutionen, sondern zentrenübergreifenden Forschungsprogrammen, die sich untereinander im Wettbewerb befinden, zur Verfügung gestellt. Eine strategische Begutachtung bildet die Basis für die Finanzierung der Forschungsprogramme. Diese Aufgabe übernehmen renommierte Experten aus aller Welt. Ihre

basis for funding decisions made by the federal and state governments and is conducted every five years. One important result of the last evaluation was the establishment of two strategic initiatives that were recommended by the panel. They concern Translational Research, which aims to speed up the process by which the results of basic research have an impact on clinical applications, and the launch of a large population study known as the "Helmholtz Cohort", which will take place over the next two decades, with the aim of improving prevention and early detection of major diseases.

The first funding period ran from 2003 to 2008. The second program period will likewise run five years, beginning in 2009 or 2010 (depending on the time of the evaluation). Evaluations for the second period began in spring 2008 (Transport and Space, Health, Earth and Environment) and in spring 2009 (Energy, Structure of Matter, and Key Technologies).

Evaluation of the centers

The MDC was extremely successful in all of its programs in the second POF evaluation, receiving the highest marks from the evaluators. The results impressively confirm the results of the evaluation of the centers from October 2006.

In addition to program evaluations, evaluations of the Helmholtz centers at the research group level are conducted. The last review of the MDC research groups was conducted in October 2006 and confirmed the positive development of the institute. Sixteen (16) international and well-renowned experts spent two days reviewing the research groups at the MDC. The results of the evaluation report were overwhelmingly positive, the reviewers clearly impressed by the MDC's achievements. Eighty-three percent (83%) of the 40 research groups received top-ratings: either "Excellent" (48%) or "Outstanding" (35%), respectively. In addition, the reviewers ranked the MDC among on of the top non-university research centers in the area of life sciences in Germany. The MDC is unique as it hosts three research programs (cardiovascular diseases, cancer, and neurosciences), allowing MDC scientists to transcend traditional boundaries and study the molecular causes of diseases in an interdisciplinary way.

Gutachten bilden die Grundlage für die Entscheidung, in welcher Höhe und in welcher Aufteilung Bund und Länder die einzelnen Programme fördern. Ein weiteres Ergebnis des letzten Begutachtungsprozesses sind zwei programmübergreifende strategische Initiativen, die auf Empfehlung der Gutachter gefördert werden. Dies sind die Translationale Forschung, um Ergebnisse aus der Grundlagenforschung schneller für die klinische Anwendung zu nutzen, sowie der Aufbau einer großen Populationsstudie „Helmholtz-Kohorte" über die kommenden zwei Jahrzehnte, um Vorbeugung und Frühdiagnostik bei den großen Volkskrankheiten zu verbessern.

Die erste Förderperiode lief von 2003 bis 2008. Die zweite Programmperiode wird ebenfalls fünf Jahre betragen, beginnend ab 2009 bzw. 2010, abhängig vom Zeitpunkt der jeweiligen Begutachtung. Die Begutachtungen für die zweite Förderperiode erfolgten im Frühjahr 2008 (Verkehr und Weltraum, Gesundheit, Erde und Umwelt) und im Frühjahr 2009 (Energie, Struktur der Materie, Schlüsseltechnologien).

Begutachtung der Zentren

Das MDC hat in allen drei Programmbegutachtungen im Rahmen der zweiten Förderperiode äußerst erfolgreich abgeschnitten und Höchstnoten von den Gutachtern erhalten. Die Ergebnisse dieser Begutachtungen bestätigten eindrucksvoll das Ergebnis der Zentrumsbegutachtung vom Oktober 2006.

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 Oktober 2006 begutachtet. Dabei konnte die positive Entwicklung des MDC eindrucksvoll bestätigt werden. 16 Experten aus dem In- und Ausland haben an zwei Tagen die Arbeiten aller Forschungsgruppen des MDC evaluiert und sich von den wissenschaftlichen Leistungen des MDC beeindruckt gezeigt. Von den insgesamt 40 begutachteten Forschungsgruppen des MDC wurden insgesamt 48 %, also knapp die Hälfte, als „excellent", und 35% der Gruppen als „outstanding" ("herausragend") eingestuft. Gleichzeitig hoben die Gutachter hervor, dass das MDC als eines der größten außeruniversitären Forschungszentren auf dem Gebiet der Lebenswissenschaften in Deutschland auch zu den erfolgreichsten zählt. Die besondere Ausrichtung des MDC mit seinen Forschungsschwerpunkten Herz-Kreislaufforschung, Krebsforschung und Neurowissenschaften macht es nach Auffassung der Gutachter möglich, die molekularen Ursachen krankheitsübergreifend zu verstehen.

The Berlin-Buch Campus

Der Campus Berlin-Buch

Biotechnology Park with Innovation and Founders' Center

The basic and clinical research that takes place on Campus Berlin-Buch and the potential of the neighboring hospitals provide a stimulating environment for the development of the BiotechPark with its Innovation and Founders' Center (IGZ). The BiotechPark along with the IGZ have developed into one of the largest such centers in Germany. To date, more than 60 million euros have been invested in the expansion and modernization of the campus infrastructure, the shared facilities, and the biotechnology park. This sum includes GA funds from the program "Joint task – improvement of the regional economic structure" and ERDF funds (European Regional Development Funds). Overall, since the mid-nineties, the investment volume of the companies located on campus has amounted to approximately 180 million euros.

Around 50 small and middle-sized companies, 38 of which are in the biomedical sector, are currently engaged in research and production in the BiotechPark. Approximately 26,000 square meters of rental commercial space are provided for the young firms, which altogether have about 750 employees. Current occupancy is at 86%. During the reporting period, two new companies moved to the BiotechPark. In addition, a number of existing companies expanded, such as Bavarian Nordic, Glycotope, Celares and Invitek. The increase in the number of firms choosing to locate in the BiotechPark is an indication of the attractiveness of Campus Berlin-Buch as a preferred location.

In its role as operation and development company for the Campus Berlin-Buch, BBB Management GmbH continued and expanded its networking activities on all levels in 2006. As part of BBB's endeavor to support campus companies and strengthen the image of the location, Campus Berlin-Buch was showcased at numerous national and international events and fairs. Event programs were organized and carried out for 26 visitor groups comprising representatives from science, business, politics and the media as well as for individual visitors from 11 countries. To promote sustained campus development, BBB worked intensively to heighten the profile of Berlin-Buch as a Health Region. BBB Management GmbH Campus Berlin-Buch

Biotechnologiepark mit Innovations- und Gründerzentrum

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 mit IGZ hat sich zu einem der Größten inDeutschland entwickelt. In den Ausbau und die Modernisierung derCampusinfrastruktur, der Gemeinschaftseinrichtungen des Campus undin den Biotechnologiepark sind bislang mehr als 60 Millionen Euroinvestiert worden. Die Fördermittel kommen aus der Gemeinschaftsaufgabe zur Verbesserung der regionalen Wirtschaftsstruktur (GA) und ausdem Europäischen Fonds für Regionale Entwicklung (EFRE). Die angesiedelten Unternehmen selbst haben seit Mitte der 90´er Jahre rund 180 Millionen Euro investiert.

Im BiotechPark forschen und produzieren auf einer Fläche von rund 26.000 Quadratmetern gegenwärtig 50 kleine und mittelständige Unternehmen. 38 davon sind im Bereich der Biomedizin tätig. Die Firmen beschäftigen etwa 750 Mitarbeiter. Zwei Unternehmen siedelten sich neu an. Die aktuelle Auslastung im BiotechPark liegt bei etwa 86%. Die gewachsene Attraktivität des Campus zieht insbesondere externe Ansiedlungen an. Zugleich expandierte eine Reihe von ansässigen Firmen des Campus, wie Bavarian Nordic, Glycotope, Celares und Invitek.

Als Betreiber- und Entwicklungsgesellschaft des Campus hat die BBBManagement GmbH im Jahr 2009 die Netzwerkarbeit auf allen Ebenenzur Unterstützung von Unternehmen und zur Stärkung des Standorts fortgeführt und ausgeweitet. Der Standort wurde erneut international und national auf Messen und Veranstaltungen präsentiert. Für 26 Besuchergruppen mit Vertretern aus Wissenschaft, Wirtschaft, Politik und Medien sowie Besuchern aus 11 Ländern wurden Veranstaltungsprogramme organisiert und betreut. Zugunsten einer nachhaltigen Campusentwicklung hat sich die BBB erneut intensiv in die weitere Profilierung des Stadtteils Berlin-Buch zu einer Gesundheitsregion eingebracht.

Die BBB Management GmbH Campus Berlin-Buch ist eine Gründung desMax-Delbrück-Centrums für Moleku-

was founded by the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch. The Institute for Molecular Pharmacology (FMP) as well as Bayer Schering Pharma are co-shareholders (20% each).

Life Science Learning Lab

In 2009 the Life Science Learning Lab (Gläsernes Labor) celebrated its tenth anniversary. This year more than 12,500 middle school and high school students, teachers, technical assistants and scientists as well as members of the general public visited lab courses and further education programs on topics such as methods and applications of genetic research, cell biology and molecular medicine. These activities are supported significantly by the MDC and other campus institutions.

The activities of the Life Science Learning Lab focused on the development of new lab courses relating to research topics of the MDC, the establishment of new lab courses in the MDC student lab and the encouragement of socially disadvantaged students. As part of the German Children and Youth Foundation project "Schools prepare for the future," the Life Science Learning Lab developed school lessons and experiments in biology, health education and nutrition for the local Hufeland school.

Central activities of the CampusPR/Life Science Learning Lab team included the editing and publication of the newsletter “CampusNews”, the publication of booklets and flyers for location marketing as well as the successful realisation of events such as the New Year’s reception and “Long Night of the Sciences," a strong magnet for visitors.

In the campus kindergarten “CampusSterne”, which was initiated by the CampusPR, all 30 places are completely booked and just three years after opening the kindergarten is going to be enlarged in 2010.

Leibniz-Institut für Molekulare Pharmakologie (FMP)

The Leibniz-Institut für Molekulare Pharmakologie (FMP) conducts basic research in the field of molecular pharmacology. Due to the close spatial proximity to

lare Medizin (MDC) Berlin-Buch. Mitgesellschafter sind das Leibniz-Institut für Molekulare Pharmakologie (FMP) sowie die Bayer Schering Pharma AG.

Das Gläserne Labor

Das Gläserne Labor hat als Life Science-Bildungszentrum im Jahre 2009 mehr als 12.500 Schülerinnen und Schülern, Lehrkräften und einem breiten Laien- und Fachpublikum anspruchsvolle Laborkurse und Fortbildungen zu aktuellen Methoden und Anwendungen der Genforschung, Zellbiologie und molekularen Medizin angeboten. Das MDC und andere Campuseinrichtungen haben diese Aktivitäten maßgeblich unterstützt.

Die Bildungsarbeit des Gläsernen Labors fokussierte sich u. a. dabei auf die Entwicklung neuer Laborkurse mit Bezug zu Forschungsthemen des MDC, auf den Ausbau der Kursangebote im MDC-Schülerlabor sowie die Förderung von sozial benachteiligten Schülerinnen und Schülern. Als Teil des Verbundprojekts »Die Schule macht fit für die Zukunft« der Deutschen Kinder- und Jugendstiftung entwickelte das Gläserne Labor geeignete Unterrichtseinheiten und Experimente zum Thema Biologie, Gesundheit und Ernährung für die an den Campus angrenzende Hufeland-Hauptschule. Zu den zentralen Aktivitäten der Campus-PR/Gläsernes Labor zählte wie in den Vorjahren die Gestaltung und Herausgabe der Mitarbeiter und Imagezeitung „CampusNews“, die Publikation von Broschüren zum Standortmarketing des Campus sowie die Organisation und erfolgreiche Durchführung von Veranstaltungen wie dem Neujahrsempfang der Campus-Einrichtungen und der äußerst erfolgreichen und besucherstarken Langen Nacht der Wissenschaften.

Die von der Öffentlichkeitsarbeit des Campus initiierte und vor drei Jahren eröffnete betriebsnahe Kindertagesstätte »CampusSterne« ist mit 30 verfügbaren Plätzen ausgebucht. Weitere Kitaplätze werden seitens der Beschäftigten des Campus nachgefragt. Für 2010 ist eine räumliche Erweiterung der Kita vorgesehen.

Leibniz-Institut für Molekulare Pharmakologie (FMP)

Das Leibniz-Institut für Molekulare Pharmakologie (FMP) betreibt Grundlagenforschung auf dem Gebiet

the MDC, the existing collaborations between the two institutes have been considerably intensified. The research concepts of the MDC and the FMP complement each other: while the molecular medical research at the MDC is particularly dedicated to diseases or clinical symptoms and their molecular explanations, the FMP investigates the functional and structural characterization of proteins. As a result, new approaches are developed toward the modulation of protein function, especially of small molecules. Hence, a main emphasis of research is the development of new drugs and pharmaceuticals. The FMP is known for its scientific work that combines the fields of chemistry and biology.

The close connection between the two research establishments extends into the organizational level. Guest scientist contracts make it possible for scientists of one institute to use the equipment in the other. Both establishments send representatives to important committees of the other establishment respectively. The planning of costly and long-term research projects as well as the appointment of leading scientists takes place in joint agreement. The MDC and the FMP arrange and finance joint events for those studying for their doctorates.

der molekularen Pharmakologie. Begünstigt durch die räumliche Nähe auf dem Campus Berlin-Buch bestehen intensive Forschungskooperationen zwischen MDC und FMP. Ihre Forschungskonzepte ergänzen sich: Während sich die molekular-medizinische Forschung des MDC besonders mit Krankheiten oder klinischen Symptomen und ihren molekularen Grundlagen beschäftigt, stehen im Mittelpunkt der FMP-Forschung der Aufbau und Funktion von Proteinen als Grundbausteine des Körpers. Ziel ist es, Wirkstoffe zu finden, die an Proteine binden und deren Funktionen ändern können. Sie kommen dann als Werkzeuge für die Forschung sowie als Bausätze für neue Arzneimittel in Frage.

Gastwissenschaftlerverträge machen es möglich, dass Wissenschaftler des einen Instituts Geräte des anderen verwenden. Beide Einrichtungen entsenden Vertreter in die wichtigsten Gremien der jeweils anderen Einrichtung. Kostspielige, langfristige Forschungsprojekte werden gemeinsam geplant, führende Wissenschaftlern in gegenseitigem Einvernehmen ernannt. Auch organisieren und finanzieren MDC und FMP gemeinsam Veranstaltungen für ihre Promotionsstudenten.

Organizational Structure

Organisationsstruktur

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 application.

Board of Trustees

The Board of Trustees is the supervisory body of MDC and monitors the conduct of operations with respect to legality, appropriateness, economic efficiency and financial viability. It decides upon general research objectives as well as important research policy and financial matters of the Foundation.

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Das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch ist eine Stiftung des öffentlichen Rechts des Landes Berlin mit dem Zweck, medizinische Forschung auf molekularer und zellulärer Ebene und ihre klinische Anwendung und praktische Umsetzung zu betreiben.

Das Kuratorium

Das Kuratorium ist das Aufsichtsgremium des MDC. Es überwacht die Rechtmäßigkeit, Zweckmäßigkeit und Wirtschaftlichkeit der Geschäftsführung. Es entscheidet über die allgemeinen Forschungsziele und über wichtige forschungspolitische und finanzielle Angelegenheiten der Stiftung.

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Institut für Genetik der Universität zu Köln*

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Dr. Matthias Selbach (ab Mai 2009)
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Prof. Dr. Mathias Hentze (ab Oktober 2009)
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Prof. Dr. Elisabeth Knust (seit August 2009)
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Scientific Committee

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 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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Prof. Dr. Anna Dominiczak (since September 2007)
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Prof. Dr. Christoph Huber
Universität Mainz

Prof. Dr. Thomas Meitinger (to May 2009)
Forschungszentrum für Umwelt und Gesundheit (GSF), Neuherberg

Der Wissenschaftliche Ausschuss

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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The Executive Board manages the Institute and consists of a scientific member, Prof. Walter Birchmeier, and an administrative member, Dr. Stefan Schwartze. The Chair of the Executive Board is Prof. Dr. Walter Birchmeier.

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The Scientific Council advises the Executive Board in matters of fundamental scientific importance.

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Prof. Dr. Elisa Izaurralde (seit August 2009)
Max Planck Institut für Entwicklungsbiologie*

Prof. Dr. Elisabeth Knust (seit August 2009)
Max Planck Institut für Molekulare Zellbiologie und Genetik*

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Der Stiftungsvorstand leitet das Institut und besteht aus einem wissenschaftlichen Mitglied, Prof. Walter Rosenthal, und einem administrativen Mitglied, Cornelia Lanz. Vorsitzender des Stiftungsvorstands ist Prof. Dr. Walter Rosenthal

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Der Wissenschaftliche Rat berät den Stiftungsvorstand in den Angelegenheiten von grundsätzlicher wissenschaftlicher Bedeutung.

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Prof. Dr. Nikolaus Rajewsky
Prof. Dr. Fritz G. Rathjen
Dr. Armin Rehm
Dr. Frank Rosenbauer
Prof. Dr. Claus Scheidereit
Prof. Dr. Peter Schlag
Dr. Ruth Schmidt-Ullrich
Dr. Björn Christian Schroeder
Dr. Anja Schütz
Dr. Matthias Selbach
Dr. Jan-Erik Siemens
Prof. Dr. Thomas Sommer
Dr. Francesca Spagnoli
Prof. Dr. Ludwig Thierfelder
Prof. Dr. Erich Wanker
Prof. Dr. Thomas Willnow
Dr. Jana Wolf
Dr. Ulrike Ziebold
Prof. Dr. Frauke Zipp

Prof. Dr. Norbert Hübner
Dr. Inés Ibanez-Tallon
Dr. Zsuzsanna Izsvák
Prof. Dr. Thomas Jentsch
Prof. Dr. Helmut Kettenmann
Dr. Markus Landthaler
Dr. Stefan Lechner
Prof. Dr. Young-Ae Lee
Prof. Dr. Ferdinand le Noble
Prof. Dr. Achim Leutz
Prof. Dr. Gary R. Lewin (Vorsitzender)
PD Dr. Martin Lipp
Prof. Dr. Friedrich C. Luft
PD Dr. Jochen Meier
Prof. Dr. Ingo L. Morano
Dr. Thomas Müller
Prof. Dr. Thoralf Niendorf
Dr. Cristiane Nolte
Dr. Matthew N. Poy
Prof. Dr. Nikolaus Rajewsky
Prof. Dr. Fritz G. Rathjen
Dr. Armin Rehm
Dr. Frank Rosenbauer
Prof. Dr. Claus Scheidereit
Prof. Dr. Peter Schlag
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Prof. Dr. Ludwig Thierfelder
Prof. Dr. Erich Wanker
Prof. Dr. Thomas Willnow
Dr. Jana Wolf
Dr. Ulrike Ziebold
Prof. Dr. Frauke Zipp

Staff Council

The Staff Council is involved in decisions of the MDC Berlin-Buch that concern personnel and staff welfare matters.

Members of the Staff Council

Ingo Kahl (Chair)
Carola Bernert (vice chair)
Lutz Else (vice chair)
Dagmar Gerhard (vice chair)
Manuela Adloff
Gitta Blendinger
Dr. Oliver Daumke
Robby Fechner
Daniela Keyner
Siegne Knespel
Jan Timm
Brigitta Wedekind (secr.)

Women's Representative

The women's representative is responsible for matters and measures concerning equal opportunities for women at the MDC. She advises not only in the planning but also in the decisions of the Board and other organizational units, in particular with regard to personnel, welfare, and organizational decisions. Currently, Dr. Hannelore Haase serves as the MDC women's representative.

Personalrat

Der Personalrat ist an Entscheidungen des MDC Berlin-Buch beteiligt, welche die personellen und sozialen Belange der Beschäftigten betreffen.

Mitglieder des Personalrates

Ingo Kahl (Vorsitz)
Carola Bernert (stellv. Vorsitz)
Lutz Else (stellv. Vorsitz)
Dagmar Gerhard (stellv. Vorsitz)
Manuela Adloff
Gitta Blendinger
Dr. Oliver Daumke
Robby Fechner
Daniela Keyner
Siegne Knespel
Jan Timm
Brigitta Wedekind (Sekr.)

Frauenvertreterin

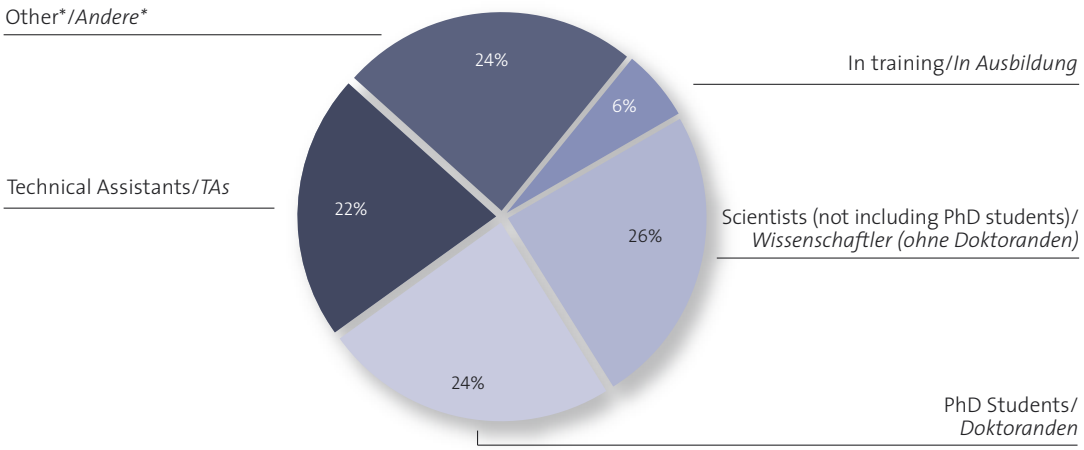
Die Frauenvertreterin ist für Angelegenheiten und Maßnahmen der Gleichstellung der Frauen am MDC zuständig. Sie wird sowohl bei der Planung als auch bei Entscheidungen des Vorstands und anderer Organisationseinheiten, insbesondere bei personellen, sozialen und organisatorischen Entscheidungen beratend beteiligt. Derzeit nimmt Dr. Hannelore Haase die Funktion der Frauenvertreterin am MDC wahr.

Facts and Figures
Fakten und Kennzahlen

Personnel / *Personal*

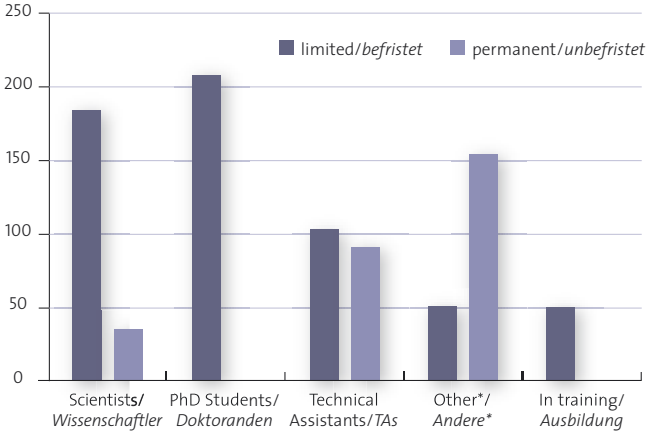
Personnel (as of December 31, 2008) / *Personalstand in Köpfen mit Stichtag 31.12.2008*

	Total/ *Gesamt*	limited contract/ *befristet*	permanent/ *unbefristet*	in-house financing/ *grundfinanziert*	third-party financing/ *drittmittelfinanziert*
Scientists (not including PhD students) / *Wissenschaftler (ohne Doktoranden)*	215	182	33	122	93
PhD students / *Doktoranden und studentische Hilfskräfte*	211	211	0	79	132
Technical Assistants (TAs) in the scientific sphere / *Technische Angestellte im wissenschaftlichen Bereich*	190	103	87	129	61
Other / *Andere*	213	52	161	197	16
In training / *In Ausbildung*	51	51	0	51	0
Total / *Summe*	880	599	281	578	302



Type of Employment Contract / *Art der Verträge*

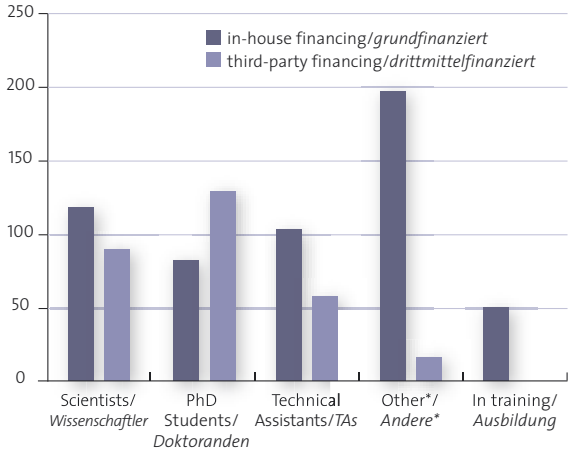
	limited/*befristet*	permanent/*unbefristet*
Scientists/*Wissenschaftler*	182	33
PhD Students/*Doktoranden.*	211	0
Technical Assistants/*TAs*	103	87
Other*/*Andere**	52	161
In training/*Ausbildung*	51	0



*Infrastructure, administration, and technology transfer
**Infrastruktur, Verwaltung und Technologietransfer*

Type of Financing/*Art der Finanzierung*

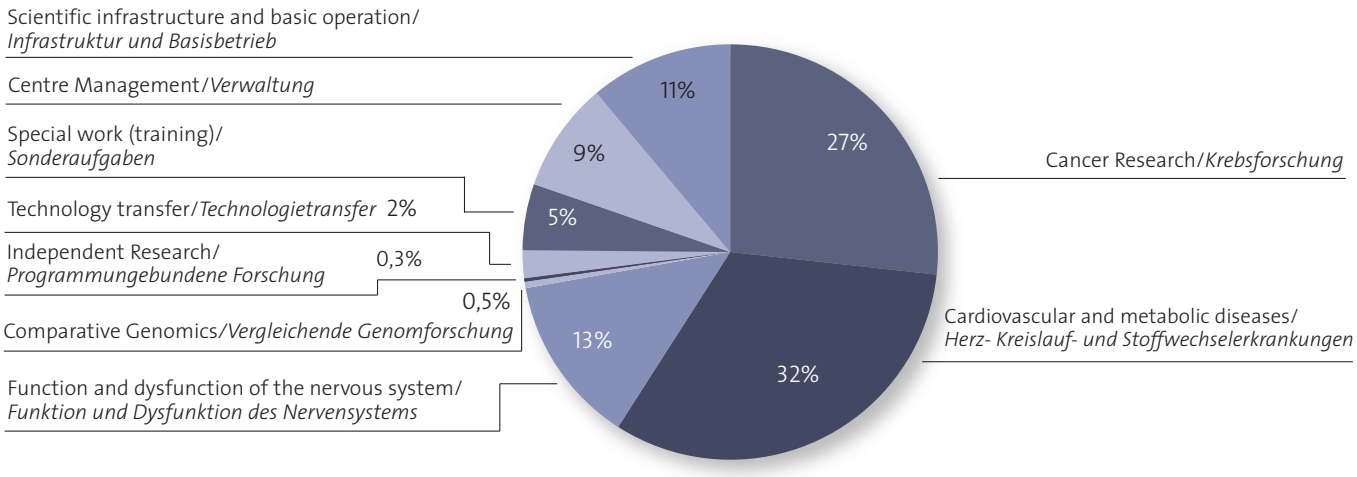
	in-house financing/ *grundfinanziert*	third-party financing/ *drittmittelfinanziert*
Scientists/*Wissenschaftler*	122	93
PhD Students/*Doktoranden*	79	132
Technical Assistants/*TAs*	129	61
Other*/*Andere**	197	16
In training/*Ausbildung*	51	0



Financing / *Finanzierung*

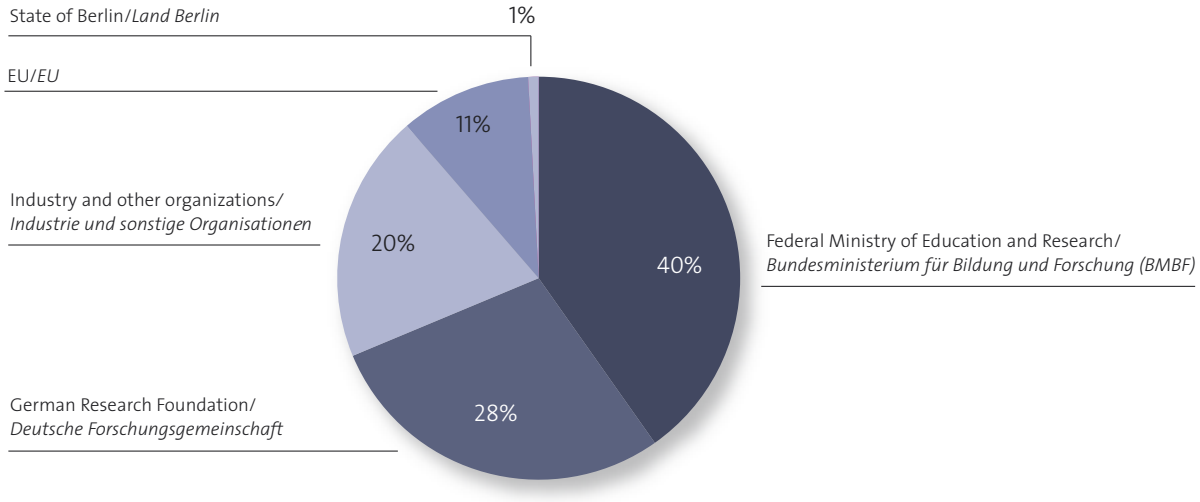
Costs of research programs in 2008 (in thousands of €) / *Kosten der Forschungsprogramme 2008 in T€*

Programs & Categories / *Programme & Kategorien*	Costs / *Kosten*
Cancer Research/*Krebsforschung*	13.806
Cardiovascular and metabolic diseases/*Herz- Kreislauf- und Stoffwechselerkrankungen*	16.696
Function and dysfunction of the nervous system/*Funktion und Dysfunktion des Nervensystems*	6.791
Comparative Genomics/*Vergleichende Genomforschung*	259
Independent Research/*Programmungebundene Forschung*	134
Technology transfer/*Technologietransfer*	1.116
Special work (training)/*Sonderaufgaben*	2.666
Centre Management/	4.427
Scientific infrastructure and basic operation/	5.745
Total/*Gesamt*	**51.640**



Extramural funding in 2008 (in thousands of €) / *Drittmittelfinanzierung 2008 in T €*

Extramural funds / *Drittmittelgeber*	Amounts / *Drittmittelerlöse*
German Research Foundation/*Deutsche Forschungsgemeinschaft*	6.138
EU/*EU*	2.266
Industry and other organizations/*Industrie und sonstige Organisationen*	4.295
Federal Ministry of Education and Research/*Bundesministerium für Bildung und Forschung (BMBF)*	8.665
State of Berlin/*Land Berlin*	181
Total / *Gesamt*	**21.545**



Research Projects 2008-2009

Forschungsprojekte 2008-2009

Collaborative research centres (SFB) und transregional research centres (TRR) of the German Research Foundation (DFG)

SFB 449:	Structure and function of membrane receptors
SFB 594:	Molecular machines in protein folding and protein transport
SFB 633:	Induction and modulation of T cell-imparted Immunreactions in the gastrointestinal tract
SFB 650:	Cellular approaches to the suppression of undesirable immunroactions
SFB 577:	Molecular principles of the clinical variability of monogenic diseases
SFB 618:	Theoretic biology: robustness, modularity and evolutionary design of living systems
SFB 665:	Development disorders in the nervous system
SFB 740:	From molecules to modules: Organization and dynamics of functional units in cells
SFB 555:	Complex non-linear processes
SFB Transregio 19:	Inflammatory cardiomyopathy – molecular pathogenesis and therapy
SFB Transregio 36:	Principles and applications of adoptive T cell therapy
SFB Transregio 43:	The brain as a target of inflammatory processes
SFB Transregio 54:	Growth and survival, plasticity und cellular interactivity of lymphatic neoplasias
SFB Transregio 52:	Transcriptional programming of individual T-cell populations
SFB Transregio 3:	Mesial temporal lobe epilepsies
SFB Transregio 3:	Mesiale Temporallappen-Epilepsien

Sonderforschungsbereiche (SFB) und Transregios (TRR) der Deutschen Forschungsgemeinschaft (DFG)

SFB 449	*Struktur und Funktion membranständiger Rezeptoren*
SFB 594	*Molekulare Maschinen in Proteinfaltung und Proteintransport*
SFB 633	*Induktion und Modulation T-zellvermittelter Immunreaktionen im Gastrointestinaltrakt*
SFB 650	*Zelluläre Ansätze zur Suppression unerwünschter Immunreaktionen*
SFB 577	*Molekulare Grundlagen klinischer Variabilität monogen bedingter Krankheiten*
SFB 618	*Theor. Biologie: Robustheit, Modularität und evolutionäres Design lebender Systeme*
SFB 665	*Entwicklungsstörungen im Nervensystem*
SFB 740	*Von Molekülen zu Modulen: Organisation und Dynamik zellulärer Funktionseinheiten*
SFB 555	*Komplexe nichtlineare Prozesse*
SFB Transregio 19	*Inflammatorische Kardiomyopathie – Molekulare Pathogenese und Therapie*
SFB Transregio 36	*Grundlagen und Anwendung adoptiver T-Zelltherapie*
SFB Transregio 43	*Das Gehirn als Angriffsziel inflammatorischer Prozesse*
SFB Transregio 54	*Wachstum und Überleben, Plastizität und zelluläre Interaktivität lymphatischer Neoplasien*
SFB Transregio 52	*Transkriptionelle Programmierung individueller T-Zell-Populationen*
SFB Transregio 3	*Mesiale Temporallappen-Epilepsien*

National Genome Research Network (NGFN-2) Projects

Genome network cardiovascular diseases: Comparative genomics of left ventricular hypertrophy and dysfunction in hypertension

Genome network cardiovascular diseases: Functional genomics of cardiac damage in hypertension

Genome network cardiovascular diseases: Location Berlin MDC: Prevalence of Titin-mutations and identification of new disease genes in patients with familial dilative cardiomyopathy

Systematic-methodical platform "DNA", location MDC, Berlin"

Systematic-methodical platform "DNA": National genotyping platform

Systematic-methodical platform GEM: Location Berlin "Genomwide correlation analysis and association studies with 10K and 100K arrays"

Genome network neuro: Systematic gene identification und functional analysis for frequent neurologic diseases

Genome network neuro: Molecular genetic identification of activity scheduled gene configurations of genetically determined epilepsies

CancerNet: Organ Specificity of Colorectal Cancer Metastasis

Systematic-methodical platform "Protein": Verification and identification of protein-protein interactions and systematic analysis of target proteins via X-ray structure analysis, TP 7 – Structure determination

Systematic-methodical platform "Protein": Verification and identification of protein-protein interactions and systematic analysis of target proteins via X-ray structure analysis, TP 20 – Project management

Systematic-methodical platform "Protein": Verification and identification of protein-protein interactions and systematic analysis of target proteins via X-ray structure analysis, TP 8 Yeast two-hybrid protein interaction networks

Systematic-methodical platform "Protein", Location MDC Berlin: Verification and identification of protein-protein interactions and systematic analysis of target proteins via X-ray structure analysis, TP 1.1. Subcloning of full-length ORF's and cDNA-fragments into expression plasmids

Determination of genes underlying Williams-Beuren Syndrom by generation of an allelic series of mutations with the aid of novel transposon approaches, TP1

Projekte im Nationalen Genomforschungsnetz (NGFN-2)

Genomforschungsnetz Kardiovaskuläre Krankheiten: Vergleichende Genomics der links-ventrikulären Hypertrophie and Dysfunktion bei Bluthochdruck

Genomforschungsnetz Kardiovaskuläre Krankheiten: Funktionelle Genomics der Herzschädigung bei Bluthochdruck

Genomforschungsnetz Kardiovaskuläre Krankheiten: Standort Berlin MDC: Prävalenz von Titin-Mutationen und Identifizierung neuer Krankheitsgene in Patienten mit Familiärer Dilativer Kardiomyopathie

Systematisch-Methodische Plattform "DNA", Standort MDC, Berlin"

Systematisch-Methodische Plattform "DNA": Nationale Genotypisierungsplattform

Systematisch-methodische Plattform GEM: Standort Berlin "Genomweite Kopplungsanalyse und Assoziationsstudien mit den 10K und 100K Chips"

Genomnetz Neuro: Systematische Genidentifikation und funktionelle Analysen bei häufigen neurologischen Erkrankungen

Genomnetz Neuro: Molekulargenetische Identifizierung von disponierenden Genkonfigurationen bei genetisch determinierten Epilepsien

CanerNet: Organspezifität von Darmkrebs Metastasierung

Systematisch-Methodische Plattform "Protein": Vertifikation und Identifikation von Protein-Protein Interaktionen und systematische Analyse von Targetproteinen mittels Röntgenstrukturanalyse, TP 7 – Struktur- aufklärung

Systematisch-Methodische Plattform "Protein": Vertifikation und Identifikation von Protein-Protein Interaktionen und systematische Analyse von Targetproteinen mittels Röntgenstrukturanalyse, TP 20 – Projekt- management

Systematisch-Methodische Plattform "Protein": Vertifikation und Identifikation von Protein-Protein Interaktionen und systematische Analyse von Targetproteinen mittels Röntgenstrukturanalyse, TP 8 Yeast two-hybrid Protein Interaktionsnetzwerk

Systematisch-Methodische Plattform "Protein", Standort MDC Berlin: Verifikation und Identifikation und systematische Analyse von Targetproteinen mittels Röntgenstrukturanalyse", TP 1.1. Umklonierung von full-length ORF's und cDNA-Fragmenten in Expressions-Plasmide

Bestimmung der Gene, die dem Williams-Beuren Syndrom zugrundeliegen durch die Generation einer allelischen Serie von Mutationen mit Hilfe von neuartigen Transposon Ansätzen, TP1

National Genome Research Network (NGFN-Plus) Projects

Network genetics of heart failure: TP3 Regulatory networks of susceptability genes for cardial hypertrophy and heart failure

Network neurodegeneration (NeuroNet): Data integration and generation of phenotype-protein-active agent networks

Network neurodegeneration (NeuroNet): Protein Interaction screening via quantitative mass spectroscopy

Network neurodegeneration (NeuroNet): Protein-protein interaction networks for neurodegenerative diseases

Network neurodegeneration (NeuroNet): Generation and systematic analysis of protein-protein interaction networks via Yeast-2-Hybrid quantitative proteomics

Network neurodegeneration (NeuroNet): Generation of Gene expression signatures for neurodegenerative disease processes

Network neuroblastoma: Transposon based mutagenesis screen in the mouse

Network leukemia: The role of the WNT-signal transduction pathway on the development of leukemia stem cells

Network Alzheimer: The physiological function of BACE1-is BACE1 a safe therapeutic target?

Systems biology of genetically determined diseases: Mutanome. Generation and systematical analysis of protein-protein interaction networks

Network Alzheimer: Identification and characterization of modulators of Alzheimer's disease pathogenesis

Network: Molecular cause for affective disorders and schizophrenia. MOODS (TP11)

Installation of the scientific platform "Interactom" for systematic protein interaction studies

Projekte im Nationalen Genomforschungsnety (NGFN-Plus)

Verbund Genetik des Herzversagens: TP3 Regulatorische Nektzwerke von Suszeptibilitätsgenen für kardiale Hypertrophie und Herzversagen

Verbund Neurodegeneration (NeuroNet): Datenintegration und Erstellung von Phänotyp-Protein-Wirkstoff Nektzwerken

Verbund Neurodegeneration (NeuroNet): Protein Interaktionsscreening durch quantitative Massenspektroskopie

Verbund Neurodegeneration (NeuroNet): Protein-Protein-Interaktionsnetzwerke bei neurodegenerativen Erkrankungen

Verbund Neurodegeneration (NeuroNet): Erstellung und systematische Analyse von Protein-Protein Interaktionsnetzwerken durch Yeast-2-Hybrid quantitative Proteomics

Verbund Neurodegeneration (NeuroNet): Erstellung von Genexpressions-signaturen von neurodegenerativen Krankheitsprozessen

Verbund: Neuroblastome: Transposon-basierter Mutagenese Screen in der Maus

Verbund: Leukämien: Die Rolle des WNT-Signalweges bei der Entstehung von Leukämie-Stammzellen

Verbund Alzheimer: Die physiologische Funktion von BACE1-ist BACE1 ein sicherer therapeutischer Angriffspunkt ?

Systembiologie genetisch bedingter Erkrankungen: Mutanom. Erstellung und systematische Analyse von Protein-Protein Interaktionsnetzwerken

Verbund Alzheimer: Identifikation and Charakterisierung von Modulatoren der Pathogenese von Alzheimer.

Verbund: Molekulare Ursachen bei Affektiven Störungen und Schizophrenie. MOODS (TP11)

Aufbau der wiss. Plattform "Interaktom" für systematische Protein-Interaktions-studien

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EU Projects

FP6

MEMORIES – Development, characterisation and validation of new and original models for Alzheimer's Disease'

MYORES – Multi-organismic Approach to study Normal and Aberrant Muscle Development, Function and Repair

AXON SUPPORT – Axonuclear Communication in Health and Disease

EuReGene – European Renal Genome Project: Funktionelle Charakterisierung neuronaler K-CI-Kotransporter mit Hilfe transgener Mausmodelle

EuroHear – Advances in hearing science: from functional genomics to therapies

ES-TRAP – A systematic approach to find new cancer molecules: an enhancer-trap screen to identify genes required for proliferation and differentiation in murine stem cells - Förderung einer Arbeitsgruppe

Translational and Functional Onco-Genomics: from cancer-oriented genomic screenings to new diagnostic tools and improved cancer treatment

RUBICON – Role of Ubiquitin and Ubiquitin-like Modifiers in Cellular Regulation

SPINE2-COMPLEXES – From receptor to gene: structures of complexes from signaling pathways linking immunology, neurobiology and cancer

INTACT – Identification of Novel Target Genes for Cancer Therapy

ATTACK – Adoptive engineered T-cell Targeting to Activate Cancer Killing

INNOCHEM – Innovative Chemokine-based Therapeutic Strategies for Autoimmunity and Chronic Inflammation

EUROSCA – European integrated project on spinocerebellar ataxias: Pathogenesis, genetics, animal models and therapy

EURATools – European Rat Tools for Functional Genomics

INTHER – Development and application of transposons and site-specific integration technologies as non-viral gene delivery methods for ex vivo gene-based therapies – Research costs

CIC-5 regulation and endocytosis at the renal proximal tubule or molecular bases of Dents disease (Marie Curie Intra-European Fellowship)

High throughput development of drugs for immunotherapy of (auto)immune diseases (Marie Curie – Research Training Networks)

EU Projekte

FP6

MEMORIES - Entwicklung, Charakterisierung und Validierung von neuen und ursprünglichen Modellen fuer die Krankheit Alzheimer

MYORES – Multi-Organismus Ansatz , um normale und abweichende Muskelentwicklung, Funktion und Regeneration zu untersuchen

AXON SUPPORT – Axonukleare Kommunication bei Gesundheit und Krankheit

EuReGene – Europäisches Nieren-Genomprojekt: Funktionelle Charakterisierung neuronaler K-CI-Kotransporter mit Hilfe transgener Mausmodelle

EuroHear – Fortschritt der Wissenschaft des Hörens: von der funktionellen Genomik zu Therapien

ES-TRAP – Ein systematischer Ansatz, um neue Krebsmoleküle zu finden: ein „enhancer-trap screen" zur Identifikation von Genen, die fuer Wachstum und Differenzierung muriner Stammzellen notwendig sind - Förderung einer Arbeitsgruppe

Translationale und funktionelle Onko-Genomics: von Krebs bezogenen Genom-Screenings zu neuen diagnostischen Moeglichkeiten und verbesserter Krebs-Behandlung

RUBICON – Die Rolle von Ubiquitin und Ubiquitin-ähnlichen Modifikatoren in der Zellregulation

SPINE2-COMPLEXES – Vom Rezeptor zum Gen: Struktur der Komplexe der Signalwege, die Immunologie, Neurobiologie und Krebs verbinden

INTACT - Identifikation neuer Zielgene fuer Krebstherapie

ATTACK - Adaptiver technisch veränderter T-Zell Angriff zur Zerstörung von Krebszellen

INNOCHEM - Innovative Chemokin-basierte therapeutische Strategien für Autoimmunität and chronische Entzündungen

EUROSCA – Europäisches integriertes Projekt zu Spinocerebellum Ataxien: Pathogenese, Genetik, Tiermodelle und Therapie

EURATools – Europaweite Ratten-Tools für funktionelle Genomik

INTHER – Entwicklung und Anwendung von Transposons und Technologien fuer spezifische Integration als nicht virale Transportmethoden fuer ex-vivo Gen-basierte Therapien

CIC-5 Regulation und Endocytose am proximalen Nierenkanal oder die molekularen Grundlagen der Dents Krankheit (Marie Curie Intra-Europäisches Stipendium)

Hochdurchsatz-Entwicklung von Medikamenten fuer die Immunotherapie von (Auto-) Immunerkrankungen (Marie Curie – Wissenschaftlicher Trainings-Verbund)

FP7

HGF/SF and MET in metastasis

EVONET – Evolution of gene regulatory networks in animal development

SET-DEV – Science, Ethics and Technological Responsibility in Developing and Emerging Countries

Start of projects in beginning of 2009

PERSIST – Persisting Transgenesis

INDUSTEM – Comparative stem cell research in mouse and humans

FP7

HGF/SF und MET in der Metastasenbildung

EVONET – Die Abstammung von gen-regulierenden Netzwerken in der Entwicklung von Tieren

SET-DEV – Wissenschaft, Ethik und technologische Verantwortung in Entwicklungs- und Schwellenlaendern

Projektbeginn zu Anfang 2009

PERSIST – Ueberdauernde Transgenese

INDUSTEM – Vergleichende Wissenschaft zu Stammzellen in Maus und Mensch

Technology Transfer / *Technologietransfer*

Figures for technology transfer in 2008 */ Kennzahlen zum Technologietransfer 2008*

Patent applications 2008 / *Patentanmeldungen 2008*	8
Patent rights / *Schutzrechtsbestand*	220
License agreements / *Lizenzverträge (Neuabschlüsse 2008)*	4
License revenues / *Lizenzerträge*	303 T€
R&D commissions (number) / *FuE-Aufträge (Anzahl)*	14
R&D commissions (proceeds) / *FuE-Aufträge (Erträge)*	283 T€
R&D cooperations (number) / *FuE-Kooperationen (Anzahl)*	222
R&D cooperations (proceeds) / *FuE-Kooperationen (Erträge)*	229 T€

In 2007 the MDC created a new internal program to stimulate technology transfer. The aim is to support projects that are reaching the point that they can be turned into applications and to open new career perspectives in the direction of applied research for MDC scientists. Researchers can apply for funding for their own position, that of a technician, and consumables for a maximum of 30,000 Euros per year, initially to run for a period of 18 months. An extension for a total of 36 months is possible. Proposals should aim to open possibilities for further funding from external sources, for example under the GO-Bio program, upon the end of MDC funding.

Zur Förderung des Technologietransfers hat das MDC im Jahr 2007 ein neues internes Förderprogramm, das Pre-GO-Bio-Programm, aufgelegt. Dieses Programm dient der Förderung von anwendungsnahen Projekten und eröffnet den Wissenschaftlern und Wissenschaftlerinnen des MDC eine Karriereperspektive in Richtung angewandter Forschung. Wissenschaftler und Wissenschaftlerinnen des MDC können eine Förderung für ihre eigene Stelle, eine TA-Stelle und Sachmittel bis zu 30.000 EUR pro Jahr zunächst für 18 Monate erhalten. Eine Verlängerung auf insgesamt 36 Monate ist möglich. Das beantragte Projekt sollte die Möglichkeit eröffnen, im Anschluss an die MDC-Förderung über eine Drittmittelförderung, wie z.B. GO-Bio, weiter entwickelt zu werden.

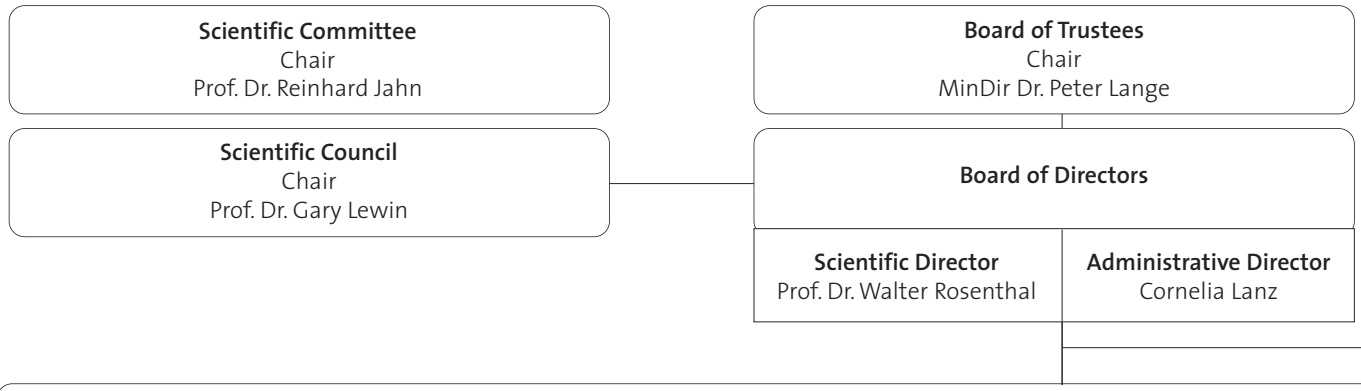
Shareholdings in companies / *Beteiligungen an Unternehmen*

Company / *Unternehmen*	Registered office / *Sitz des Unternehmens*	Homepage
BBB Management GmbH Campus Berlin-Buch	Robert-Rössle-Straße 10, 13125 Berlin	www.bbb-berlin.de
RZPD Deutsches Ressourcenzentrum für Genomforschung*	Heubnerweg 6, 14059 Berlin	www.rzpd.de
HELIOS Research Center GmbH (HRC)	HELIOS Klinikum Berlin, Karower Str. 11, Haus 214, 13125 Berlin	www.helios-kliniken.de

* The RZPD ended operations on July 31, 2007, and is in a shutting-down phase.

** Das RZPD hat zum 31.07.2007 seinen Betrieb beendet und befindet sich in der Abwicklungsphase.*

Organisation chart of the Max-Delbrück-Center for Molecular Medicine Foundation under public law



MOLECULAR MEDICINE

Cardiovascular and Metabolic Diseases *Coordinator: Prof. Dr. Thomas Willnow*

Clinical Research
Coordinator Prof. Dr. Friedrich C. Luft

Basic concepts of cardiovascular function

Prof. Dr. Thomas Willnow
Prof. Dr. Ingo L. Morano
Prof. Dr. Michael Gotthardt
Prof. Dr. Thomas Jentsch
Dr. Francesca Spagnoli
Dr. Ferdinand le Noble
Dr. Salim Seyfried
Dr. Kai Schmidt-Ott

Genetics and pathophysiology of cardiovascular diseases

Prof. Dr. Nikolaus Rajewsky
Prof. Dr. Norbert Hübner
Prof. Dr. Friedrich C. Luft
Prof. Dr. Ludwig Thierfelder
Dr. Markus Landthaler
Dr. Matthew Poy
Prof. Dr. Thoralf Niendorf

Cancer Research *Coordinator: Prof. Dr. Claus Scheidereit*

Clinical Research
Coordinator: Prof. Dr. Peter Schlag

Signaling pathways, cell biology and tumor biology

Prof. Dr. Achim Leutz
Prof. Dr. Claus Scheidereit
Prof. Dr. Clemens Schmitt
Prof. Dr. Harald Saumweber
Prof. Dr. Peter M. Schlag
Prof. Dr. Walter Birchmeier
Prof. Dr. Peter Daniel
Prof. Dr. Thomas Sommer
Dr. Ulrike Ziebold
Dr. Frank Rosenbauer

Structural and functional genomics

Prof. Dr. Udo Heinemann
Dr. Oliver Daumke

Bioethics

Dr. Christof Tannert

Function and Dysfunction of the Nervous System *Coordinators: Prof. Dr. Carmen Birchmeier-Kohler and Prof. Dr. Helmut Kettenmann*

Clinical Research
Coordinator: Prof. Dr. Frauke Zipp

Signaling pathways and mechanisms in the nervous system

Prof. Dr. Carmen Birchmeier
Prof. Dr. Jochen Meier
Prof. Dr. Gary Lewin
Prof. Dr. Fritz G. Rathjen
Dr. Inés Ibañez-Tallon
Dr. Björn Schröder
Dr. Jan Erik Siemens
Dr. James Poulet

Imaging the living brain

Prof. Dr. Frauke Zipp

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN BERLIN-BUCH

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Chair
Ingo Kahl

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Anti-Corruption Officer: Ina Herrmann
Ombudsman: Prof. Dr. Jens Reich

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Dr. Zsuzsanna Izsvák
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PD Dr. Martin Lipp
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Prof. Dr. Bernd Dörken
Prof. Dr. Antonio Pezzutto
Prof. Dr. Wolfgang Uckert
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Prof. Dr. Helmut Kettenmann
Prof. Dr. Erich Wanker

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IT
Bernd Lemke

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Construction: Ralf Streckwall
Operation: Michael Arnold

Purchasing and Central Services
Wolfgang Groll

Auditing and Legal Affairs / Technology Transfer
Christine Rieffel-Braune

Stand: November 2007

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Campus Map

Campusplan

Campus Berlin-Buch
Der Gesundheit verpflichtet

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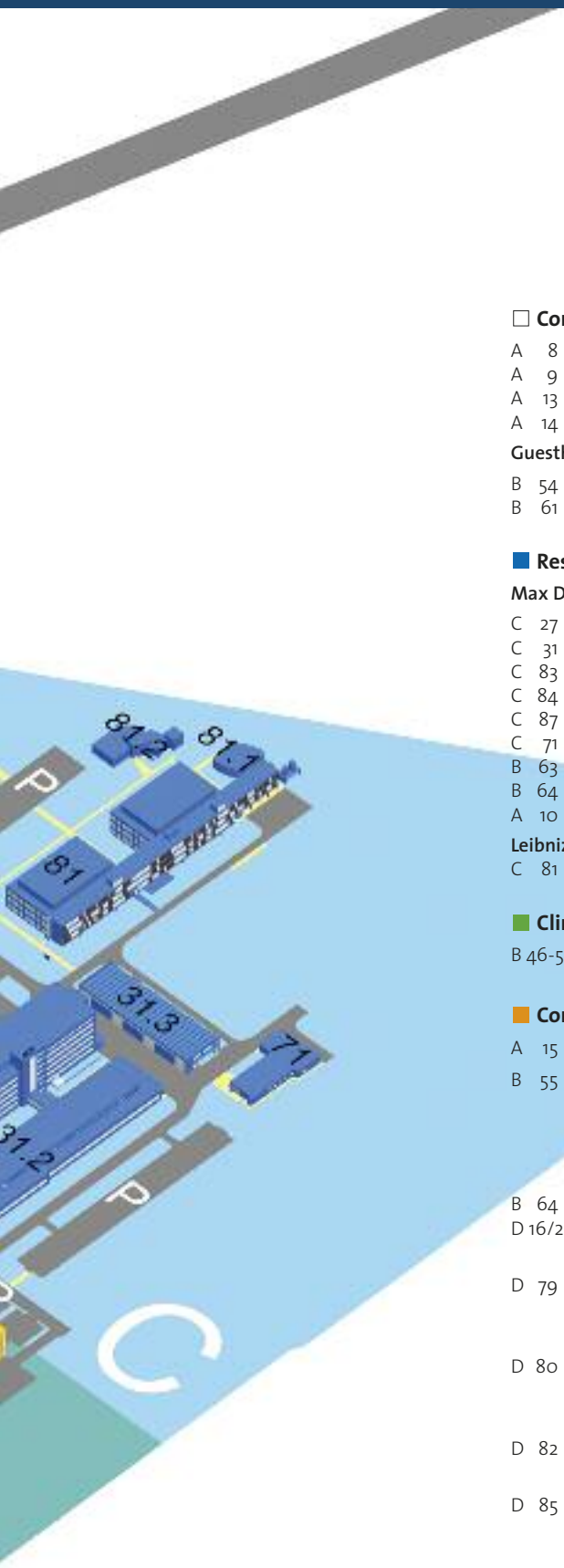

☐ Common Facilities

A 8 Gate House with Café Max and apartments
A 9 Reception
A 13 Life Science Learning Lab; CampusInfoCenter
A 14 Cafeteria

Guesthouses of the MDC

B 54 Hans-Gummel-Guest House
B 61 Kindergarden; Salvadore-Luria-Guest House

■ Research

Max Delbrück Center for Molecular Medicine (MDC)

C 27 Walter-Friedrich-House
C 31 Max-Delbrück-House
C 83 Max-Delbrück-Communications Center
C 84 Hermann-von-Helmholtz-House
C 87 Timoféeff-Ressovsky-House
C 71, B 63, B 64 } Research services
A 10 Library

Leibniz-Institut für Molekulare Pharmakologie

C 81 Leibniz-Institut für Molekulare Pharmakologie (FMP)

■ Clinical Research

B 46-51 Clinical Research

■ Companies

A 15 car mechanics, EZAG, Charles River, WISAG

B 55 **Oskar und Cécile Vogt House**
BBB Post office, patent lawyer Dr. Baumbach, FILT, ConGen, E.R.D.E., Höppner, HUMAN, Zell GmbH, TECAN, Dr. Scherrer, ART-CHEM, Roboklon, Gressus, Fresenius, 8sens.biognostic, neptuntec

B 64 epo

D 16/23 Eckert & Ziegler AG, NEMOD, Eurotope, Glykotope, BEBIG, Eckert Consult, Isotope Products

D 79 **Erwin Negelein House**
Glycotope, Isotope Products, celares, imaGenes, BioTeZ, Bavarian Nordic (House 31.1)

D 80 **Otto Warburg House**
ALRISE, Silence Therapeutics, Combinature, PolyPhag Evotec AG

D 82 **Karl-Lohmann-House**
Eckert & Ziegler, BEBIG, AJ Innuscreen

D 85 **Arnold Graffi House**
BBB, I.M.S.M., INvitek, aokin, Biosyntan, L.O.S., Clin. Research, rennesens, Prof. Wanker, MerLion, emp, Akademie der Gesundheit, Geneo BioTechProducts

How to find your way to the MDC

Der Weg zum MDC

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