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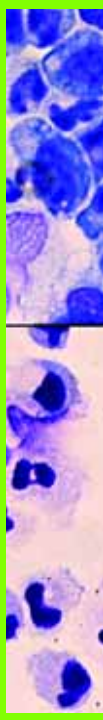

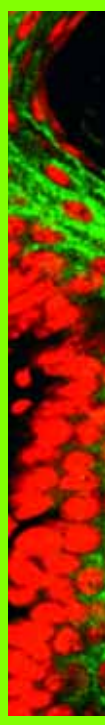

Max Delbrück Center for Molecular Medicine

MDC
Berlin-Buch

Research Report 2006
(covers the period 2004-2005)

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Berlin-Buch

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Graph representation of 3,186 putative interactions between 1,705 different human proteins (drawn using the Pajek program package). Reprinted from Cell 122 Vol. 6, Stelzl et al. A human protein-protein interaction network: a novel resource for annotating the proteome. 957-968, Copyright (2005), with permission from Elsevier.

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Covers the period 2004/2005

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Foreword

It is my pleasure to present you with the 2006 Research Report of the Max Delbrück Center for Molecular Medicine (MDC) in Berlin-Buch, which covers the research periods 2004 and 2005. Founded in 1992, the MDC is a young research institute that is sponsored by the German Federal government (90%) and the State of Berlin (10%) and is a member of the Helmholtz Association of National Research Centers *(Helmholtz-Gemeinschaft Deutscher Forschungszentren)*. In addition to federal and state funds, the MDC augments its total research budget via third-party financial resources.

Research at the MDC focuses on the molecular analysis and treatment of the most prevalent diseases in the population, namely cardiovascular diseases, cancer, and neurological diseases. Two research clinics of the Charité University Medical School in Berlin-Buch, the Franz Volhard Clinic for Cardiovascular Diseases as well as the Robert Rössle Cancer Clinic, are connected to the MDC. Regarding patient care, these two clinics are part of the HELIOS Clinics GmbH. The relationships of the MDC to the Charité's neurobiological clinics sector are being developed at present. Together with the MDC's partner institute, the Research Institute for Molecular Pharmacology *(FMP)*, and the around 30 companies on the campus in Berlin-Buch, an entire repertoire of technologies is available on the campus for characterizing diseases molecularly, identifying new starting points in diagnostics and treatment, and realizing them in clinical application. Along with genetic and cell biological characterizations of diseases, scientists in Berlin-Buch can analyze the structure of essential macromolecules and develop substances to interact with them. Based on this model, the MDC and Charité plan to establish a jointly run Experimental and Clinical Research Center (ECRC) on the Berlin-Buch campus. The ECRC will intensify the exchange of scientific ideas between the laboratory and the clinic in both directions and, hence, accelerate the transmission of scientific findings directly into clinical applications. An optimum scientific and technical environment will be created within the ECRC in which the most promising joint projects between the MDC and Charité can be conducted in

Vorwort

Es ist mir eine große Freude, Ihnen hiermit den wissenschaftlichen Bericht 2006 des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch vorzulegen, der die Forschungsperiode 2004 und 2005 umfasst. Das MDC ist ein junges Forschungsinstitut, begründet 1992, das vom Bund und dem Land Berlin im Verhältnis 90 zu 10 getragen wird und zur Helmholtz-Gemeinschaft Deutscher Forschungszentren gehört. Das MDC ist für seine Forschung stark auf die Einwerbung von Drittmitteln angewiesen, die einen großen Teil seiner effektiven Forschungsmittel ausmachen.

Das MDC beschäftigt sich wissenschaftlich mit der molekularen Analyse und Therapie der wichtigsten Volkskrankheiten, Herz-Kreislauf-, Krebs- und neurologischen Erkrankungen. Angeschlossen sind dem MDC zwei Forschungskliniken der Charité – Universitätsmedizin Berlin in Berlin-Buch, die Franz-Volhard-Klinik für Herz-Kreislauf-Erkrankungen sowie die Robert-Rössle-Krebsklinik. In der Krankenversorgung sind diese beiden Kliniken in die HELIOS Kliniken GmbH integriert. Die Beziehungen des MDC zu den Kliniken der Charité im neurobiologischen Bereich werden zur Zeit ausgebaut. Zusammen mit dem Partner-Institut des MDC, dem Forschungsinstitut für Molekulare Pharmakologie (FMP), und den rund 30 Firmen auf dem Bucher Campus steht in Berlin-Buch somit nahezu das gesamte Repertoire an Techniken zur Verfügung, um Krankheiten molekular zu charakterisieren, neue Ansatzpunkte in Diagnostik und Therapie zu identifizieren und in der klinischen Anwendung umzusetzen. Neben der genetisch/zellbiologischen Beschreibung der Krankheiten können die Bucher Wissenschaftlerinnen und Wissenschaftler die Struktur von essentiellen Makromolekülen analysieren und Substanzen entwickeln, die mit ihnen in Wechselwirkung treten. Auf dieser Grundlage planen MDC und Charité, ein gemeinsam getragenes Experimental and Clinical Research Center (ECRC) auf dem Campus Berlin-Buch zu errichten. Das ECRC wird den Austausch wissenschaftlicher Ideen zwischen Labor und Klinik in beide Richtungen verstärken und auf diese Weise die Übertragung, Translation, wissenschaftlicher Ergebnisse in die klinische Anwendung beschleunigen. Mit dem ECRC wird ein optimales wissenschaftliches und

Der damalige Bundespräsident Johannes Rau und seine Frau im Januar 2004 im Gläsernen Labor auf dem Campus Berlin-Buch. Photo: Thomas Oberländer / Copyright: Helios Klinikum Berlin-Buch
The former German President, Johannes Rau, and his wife in January 2004 in the Life Science Learning Laboratory at the Campus Berlin-Buch. Photo: Thomas Oberländer / Copyright: Helios Klinikum Berlin-Buch

the three areas of cardiovascular, cancer, and neurobiological research. In this way, the ECRC will reinforce the existing synergies on the campus and raise them to a new level.

I would like to mention a few of the many high-impact scientific publications of scientists from the MDC and its partner clinics that arose during the report period. Ludwig Thierfelder's group discovered mutations in the plakophilin 2 gene in more than 25% of patients with arrhythmic right ventricular cardiomyopathies (ARVC) (Gerull et al., 2004, *Nature Genetics*). This means that high mutation rates, which usually only occur with frequent cancer types, are now also known with cardiomyopathies. Regarding the sequence analysis of the rat genome, the group led by Norbert Hübner generated a SNP map (Single Nucleotide Polymorphism Point Mutations) of this genome (Zimdahl et al., 2004, *Science*). The comparison of the cDNAs (complementary DNA) of three different laboratory rat strains with the standard rat genome showed over 12,000 variations here. This “card” is a valuable instrument for comparative gene analysis with other mammals and can help identify medically important genes. Clemens Schmitt’s group was able to identify senescence as a new type of tumor suppressor mechanism which limits the transformation capacity of oncogenes (Braig et al., 2005, *Nature*). An inactivation of this mechanism leads to aggressive lymphomas that are apoptosis-competent. When mitogenic oncogenes are activated, a cell security program is activated that leads to either apoptosis (cell suicide) or senescence (the suspension of the cell cycle). Thomas Willnow’s group was able to explain a fundamentally new mechanism as to how sex hormones are transported to those sites where they are functionally necessary (Hammes et al., 2005, *Cell*). The steroid hormones are bound to the cell surface in the complex with their plasma transport proteins by a receptor, megalin, and transported to the nucleus. Lack of this receptor in knockout mice leads to

technisches Umfeld geschaffen werden, in dem die aussichtsreichsten Kooperationsprojekte zwischen MDC und Charité in den Schwerpunkten Herz-Kreislauf-Forschung, Krebsforschung, Neurobiologische Forschung bearbeitet werden können. Auf diese Weise wird das ECRC die bestehenden Synergien auf dem Campus verstärken und auf ein neues Niveau heben.

Ich möchte für die Berichtsperiode unter vielen wissenschaftlichen Arbeiten des MDC und seiner Partner-Kliniken in sogenannten High-Impact-Journalen einige wenige besonders erwähnen. Die Gruppe von Ludwig Thierfelder hat Mutationen im Gen Plakophilin-2 bei über 25 % der Patienten mit Arrythmischer Rechtsventrikulärer Kardiomyopathie (ARVC) gefunden (Gerull et al., 2004, *Nature Genetics*). Damit sind nun auch bei Kardiomyopathien hohe Mutationsraten bekannt, die sonst nur bei häufigen Krebsarten auftreten. Im Rahmen der Sequenzanalyse des Rattengenoms hat die Gruppe von Norbert Hübner eine SNP-Karte (Single Nucleotide Polymorphisms – Punktmutationen) dieses Genoms vorgelegt (Zimdahl et al., 2004, *Science*). Der Vergleich der cDNAs (complementary DNA) von drei verschiedenen Laborrattenstämmen mit dem „Standardgenom“ der Ratte zeigte dabei über 12.000 Variationen. Diese „Karte“ ist ein wertvolles Instrument für die vergleichende Genomanalyse mit anderen Säugern und kann helfen, medizinisch wichtige Gene zu identifizieren. Die Gruppe von Clemens Schmitt konnte Seneszenz als einen neuartigen Tumorsuppressor-Mechanismus identifizieren, der die Transformationskapazität von Onkogenen limitiert (Braig et al., 2005, *Nature*). Eine Inaktivierung dieses Mechanismus führt zu aggressiven, aber dennoch Apoptose-kompetenten Lymphomen. Bei der Aktivierung mitogener Onkogene wird ein Sicherheitsprogramm der Zelle aktiviert, das entweder zur Apoptose, Selbstmord der Zelle, oder zur Seneszenz, Stopp des Zellzyklus, führt. Die Gruppe

the inability to react to sexual hormones (steroid insensitivity) and, as a consequence, to incompletely formed sexual organs. The group led by Thomas Blankenstein was able to show that, contrary to previous knowledge, immunogenic tumors do not have to escape detection through T-cells (Willimsky und Blankenstein, 2005, *Nature*). This mainly affects so-called spontaneous tumors that develop in the absence of external influences. Erich Wanker's group generated a genome-wide interaction map of human proteins that shows 3,186 protein interactions among 1,705 proteins (Stelzl et al., 2005, *Cell*). These include previously unknown interaction partners of 195 proteins associated with diseases.

The Research Report contains three main sections reflecting the three foci of research at the MDC: cardiovascular research, cancer research, and neurological sciences. These sections are presented in English and intended for scientists and students as well as for new co-workers. Essential parts of the report are presented in German to make this report accessible to a broader public. Finally, the report provides a general overview of the MDC including a description of the newly planned ECRC, administrative structure, financial statistics, as well as currently funded scientific projects.

The MDC has been able to recruit new group leaders for the MDC (see "Academic Appointments"). The German Cancer Prize for 2005 was awarded for a second time to MDC scientists: Claus Scheidereit and Bernd Dörken. This prize had previously been awarded to Walter Birchmeier and Peter M. Schlag in 1999. A further highlight was the completion and operational start of the new animal facility and the new research building for medical genome research. Both buildings are joint projects of the MDC and the Institute for Molecular Pharmacology *(FMP)*. The medical genome research building has an area of about 3200 m² of new research space available. In this context, three W3/C4 professorships in medical genome research, bioinformatics/system biology, and cardiovascular and metabolic diseases were filled. Norbert Hübner was appointed to the new W2/C3 professorship for genetics/genomics.

We hope you enjoy reading the MDC Research Report 2006

Walter Birchmeier
Scientific Director

von Thomas Willnow konnte einen grundlegend neuen Mechanismus aufklären, wie Sexualhormone an ihre Wirkorte gelangen (Hammes et al., 2005, *Cell*). Die Steroidhormone werden im Komplex mit ihren Plasmatransportproteinen von einem Rezeptor, Megalin, auf der Zelloberfläche erkannt und in das Zellinnere transportiert. Ein Fehlen des Rezeptors in Knockout-Mäusen führt zum Unvermögen, auf Sexualhormone anzusprechen (Steroidinsensitivität) und, als Konsequenz, zu unvollständig ausgebildeten Geschlechtsorganen. Die Gruppe von Thomas Blankenstein konnte zeigen, dass entgegen bisheriger Erkenntnisse immunogene Tumore der Erkennung durch T-Zellen nicht entkommen müssen (Willimsky und Blankenstein, 2005, *Nature*). Dies trifft vor allem auf sogenannte spontane, ohne äußere Einflüsse entstandene Tumore zu. Die Gruppe von Erich Wanker hat eine genomweite Interaktionskarte von menschlichen Proteinen vorgelegt, die 3.186 Protein-Wechselwirkungen zwischen 1.705 Proteinen darstellt (Stelzl et al., 2005, *Cell*). Darunter befinden sich auch bislang unbekannte Interaktionspartner von 195 krankheits-assoziierten Proteinen.

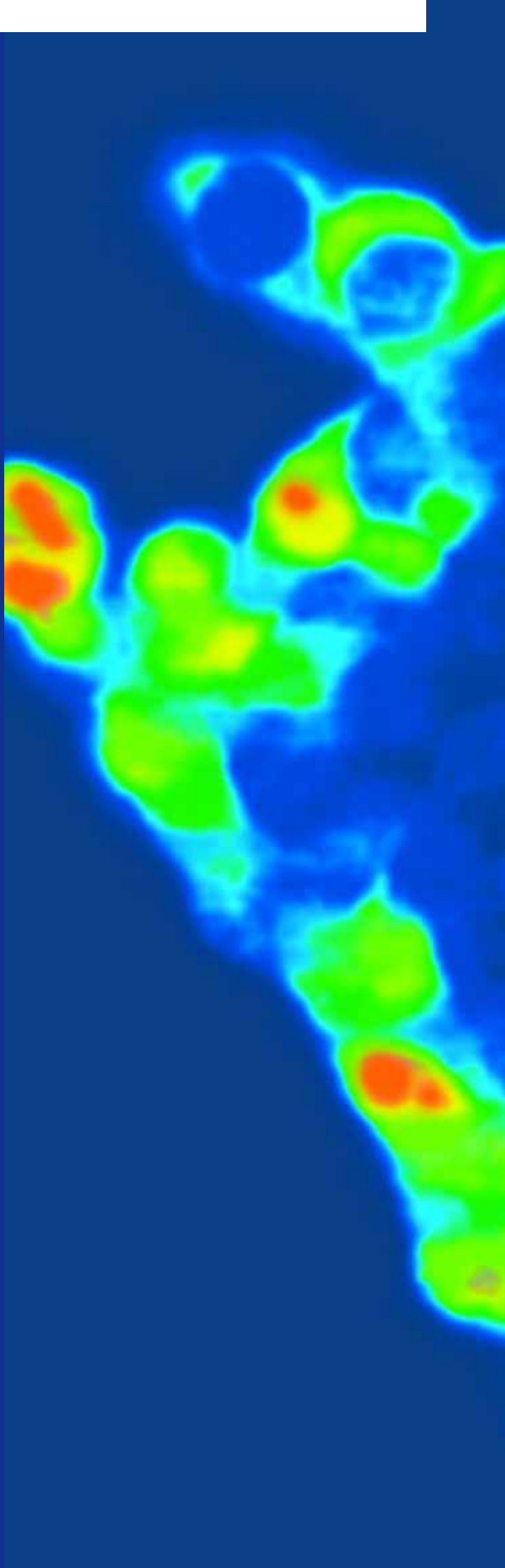
Sie sehen drei Teile im Wissenschaftlichen Bericht, die sich mit der Forschung in diesen drei Bereichen – Herz-Kreislaufforschung, Krebsforschung und Neurowissenschaften – beschäftigt. Sie sind in Englisch geschrieben und für Wissenschaftler und Studenten, auch potentielle neue Mitarbeiter, gedacht. Essentielle Teile des Berichtes sind in Deutsch verfasst, um diesen Bericht auch einer breiteren Öffentlichkeit zugänglich zu machen. Zusätzliche Teile des Berichtes beschäftigen sich mit dem geplanten ECRC sowie neuen Entwicklungen in der Verwaltung, einer Übersicht zur Finanzierung und derzeit geförderten wissenschaftlichen Projekten.

Im Berichtszeitraum konnten neue Nachwuchswissenschaftler für das MDC gewonnen werden. Der Deutsche Krebspreis ging im Jahr 2005 zum zweiten Mal an Wissenschaftler des MDC: Claus Scheidereit und Bernd Dörken wurden 2005 mit dem Deutschen Krebspreis ausgezeichnet. Schon 1999 hatten Walter Birchmeier und Peter M. Schlag diese Auszeichnung erhalten. Ein weiterer Höhepunkt war die Fertigstellung und Inbetriebnahme des neuen Tierhauses 2004 sowie des neuen Forschungsgebäudes für Medizinische Genomforschung Anfang 2006. Beide Gebäude sind Gemeinschaftsprojekte von MDC und dem Forschungsinstitut für Molekulare Pharmakologie (FMP). In dem Gebäude für Medizinische Genomforschung stehen ca. 3200 m² an neuen Forschungsflächen zur Verfügung. In diesem Zusammenhang werden eine W3/C4-Professur für Medizinische Genomforschung (gemeinsam mit dem FMP), eine W3/C4-Professur für Bioinformatik/Systembiologie sowie eine W3/C4-Professur für Herz-Kreislauf- und Stoffwechselerkrankungen neu besetzt. Auf die neu zu besetzende W2/C3-Professur für Genetik/Genomik wurde Nobert Hübner berufen.

Beim Studium dieses Forschungsberichtes wünsche ich Ihnen viel Vergnügen.

Walter Birchmeier
Wissenschaftlicher Stiftungsvorstand

Cardiovascular and Metabolic Diseases



Hypertension, Vascular Disease, and Kidney Disease
Coordinator: Thomas Willnow

Heart Disease
Coordinator: Ludwig Thierfelder

Metabolic Diseases, Genetics, Genomics, and Bioinformatics
Coordinator: Jens Reich

Cardiovascular and Metabolic Diseases Research

Michael Bader
Norbert Hübner
Friedrich Luft
Jens Reich
Ludwig Thierfelder
Thomas E. Willnow

Introduction

Diseases of the cardiovasculature and the metabolism are the major cause of morbidity and mortality in our society. Because such disorders particularly affect the elderly, the socioeconomic impact of these disease entities is expected to rise even further in aging populations of the Western world. Research in this program aims at elucidating the genes and genetic pathways that regulate the normal function of the cardiovascular system and the metabolism and that cause human disease in these areas. Ultimately, identification of disease genes will lead to a better understanding of cardiovascular disease processes, to improved diagnosis, and to new concepts in therapy.

Towards these goals, we use functional genomics approaches to study disease processes in many systems that provide utilitarian models including fruit fly, frog, mouse, and rat, and we compare our findings to studies conducted in human subjects (and vice versa). Our studies are performed by scientists that lead research groups at the MDC in close collaboration with clinicians at the Franz-Volhard-Clinic for Cardiovascular Diseases (FVK). Research activities are coordinated in three topics that are of particular relevance to this program, namely
(1) Hypertension, Vascular Disease, and Kidney Disease
(2) Heart Disease
(3) Metabolic Diseases, Genetics, Genomics, and Bioinformatics.

Herz-Kreislauf- und Stoffwechselerkrankungen

Michael Bader
Norbert Hübner
Friedrich Luft
Jens Reich
Ludwig Thierfelder
Thomas E. Willnow

Einführung

Erkrankungen des kardiovaskulären Systems und des Stoffwechsels sind die Hauptursache für Morbidität und Mortalität in unserer Gesellschaft. Auf Grund eines deutlichen Anstiegs der durchschnittlichen Lebenserwartung in unserer Bevölkerung und des erhöhten Risikos älterer Menschen, an kardiovaskulären Komplikationen zu erkranken, ist davon auszugehen, dass die Belastungen unserer Gesundheitssysteme durch die Folgekosten kardiovaskulärer Krankheiten zukünftig dramatisch ansteigen werden. Ziel unserer Forschungsanstrengungen vor diesem Hintergrund ist es, die genetischen Mechanismen aufzuklären, welche die normalen Funktionen von Herz-Kreislauf und Stoffwechsel regeln und welche für krankhafte Veränderungen dieser Systeme beim Patienten verantwortlich sind. Letztlich wird die Identifizierung grundlegender genetischer Mechanismen zu einem besseren Verständnis kardiovaskulärer Krankheitsprozesse, zu verbesserter Diagnostik und zu neuen therapeutischen Ansätzen führen.

Um dieses Ziel zu erreichen, verfolgen wir ein Konzept der vergleichenden Genomforschung, bei dem wir normale physiologische Prozesse und krankhafte Veränderungen des kardiovaskulären Systems parallel in Patienten sowie in experimentellen Tiermodellen untersuchen und miteinander vergleichen. Aus den Informationen, welche wir in Modellsystemen wie der Fruchtfliege, dem Krallenfrosch oder Nagern gewinnen, lassen sich wichtige Rückschlüsse auf relevante Krankheitsprozesse beim Menschen ziehen und neue Strategien zu deren Therapie entwickeln. Unsere Arbeiten sind das Ergebnis einer erfolgreichen Zusammenarbeit von Grundlagenwissenschaft am MDC und klinischer Forschung an der Franz-Volhard-Klinik für Herz-Kreislauferkrankungen (FVK) der Charité – Universitätsmedizin Berlin auf dem Campus Berlin-Buch. Unsere Forschungsaktivitäten konzentrieren sich auf drei Themenfelder mit besonders hoher Relevanz für kardiovaskuläre Erkrankungen:
(1) Hypertonie, Gefäß- und Nierenerkrankungen
(2) Herzerkrankungen
(3) Genetik, Genomik, Bioinformatik und Metabolismus

Hypertension, Vascular Disease, and Kidney Disease

Hypertension is a complex regulatory disorder that results in increased blood pressure. The heart, the blood vessels, and the kidney are involved either as a primary cause or as a secondary target of this disease. With the elucidation of hitherto unknown genetic mechanisms contributing to hypertension, vascular disease, and kidney disease, new therapies may become possible. In the past two years, scientists in this topic have made important contributions towards this goal.

Antibody-mediated rejection is thought to account for around one-third of all rejections following kidney transplantation. The association between HLA antibodies present at the time of transplant and graft loss has been well established. Now, Duska Dragun, Dominik Müller, Gerd Wallukat and their colleagues uncovered that a non-HLA, angiotensin II type 1 (AT1)-receptor meditated pathway may be involved in kidney transplant rejection. The scientists studied 33 kidney transplant recipients who presented with antibody-mediated rejection following kidney allograft. Of these, 13 had donor-specific anti-HLA antibodies. Sixteen of the remaining 20 patients without anti-HLA antibodies were shown to have both agonistic antibodies targeting the AT1-receptor and malignant hypertension. Furthermore, when agonistic antibodies were transferred to rats that had received kidney transplants, hypertension and vasculopathy were induced. Of the 16 patients with agonistic antibodies, seven underwent plasmapheresis for the removal of the antibodies and received the AT1-receptor blocker losartan. As compared to those control patients who received standard treatments, these seven fared better in terms of renal function and graft survival. Thus, novel therapies for the treatment of antibody-mediated rejection may be developed that involve the removal of AT1-receptor antibodies or the blockage of AT1 receptors.

Ralph Kettritz and his colleagues have elucidated central signaling pathways leading to systemic vasculitis, an inflammatory process in medium to small blood vessels caused by antibodies directed against neutrophil components. They found that integrins and cytokines activate nuclear transcription factor-kappaB in human neutrophils. They showed that beta (2) integrins provide co-stimulatory signals allowing soluble mediators to activate the NF-kappaB pathway when the cells are fixed to matrix, even when they are not capable of doing so when the cells are in suspension. This effect may become important when human neutrophils leave the circulating blood and migrate through extracellular matrix during inflammation. Michael Bader and colleagues have identified the role of the *mas* protooncogene as a receptor for angiotensin (1-7) and the cardioprotective actions of this angiotensin-II metabolite.

The group of Thomas Willnow, in collaboration with colleagues at the University of Aarhus, has uncovered the existence of endocytic pathways that govern the tissue-specific uptake of steroid hormones such as androgens and estrogens. Previously, the delivery of steroids to their respective target tissues was believed solely to depend on non-specific diffusion of the hormones through the plasma membrane. The identification of active uptake pathways for androgens and estrogens challenges a central dogma in steroid hormone biology and holds tremendous potential for therapeutic strategies

Hypertonie, Gefäß- und Nierenerkrankungen

Hypertonie, die krankhafte Erhöhung des Blutdrucks, ist eine komplexe Regulationsstörung des Kreislaufs, welche in unserer Bevölkerung weit verbreitet ist. Fehlfunktionen des Herzens, der Gefäße oder der Nieren sind primäre Ursache dieser Störung oder treten sekundär als Folge pathologischer Veränderungen beim Hypertoniker auf. Durch die Entschlüsselung bislang unbekannter genetischer Mechanismen, die zu Bluthochdruck, zu Gefäßerkrankungen oder zu Nierendefekten führen, hoffen wir die Ursachen der Hypertonie aufzuklären und neue Strategien zu deren Prävention entwickeln zu können. In den vergangenen zwei Jahren gelang es uns, wichtige neue Erkenntnisse in dieser Hinsicht zu gewinnen.

Autoantikörper-induzierte Abstoßungsreaktionen sind die Ursache für etwa 30% aller Fälle von Organverlust bei Nierentransplantation. In neueren Arbeiten konnten Duska Dragun, Dominik Müller, Gerd Wallukat und ihre Kollegen zeigen, dass aktivierende Autoantikörper gegen den Angiotensin II type 1 (AT1)-Rezeptor ursächlich für die Entstehung von Hypertonie und Nierentransplantat-Abstoßung im Patienten sind. Dies ließ sich durch den Transfer von anti-AT1-Antikörpern in Ratten dokumentieren, die dadurch eine fulminante Hypertonie und Gefäßschädigungen entwickelten. In einem Pilotexperiment konnten mittels Plasmapherese anti-AT1-Autoantikörper aus Serum von Patienten mit einer Spenderniere entfernt und damit eine signifikante Verbesserung renaler Funktionen und der Transplantat-Lebensdauer erzielt werden.

Der Arbeitsgruppe von Ralph Kettritz gelang es, zentrale Signaltransduktionsmechanismen in der Entstehung systemischer Vaskulitis, einer entzündlichen Erkrankung kleiner und mittlerer Gefäße, aufzuklären. Dieser Krankheitsprozess beruht auf einer Immunreaktion des Körpers gegen zelluläre Bestandteile von Neutrophilen. Die Wissenschaftler konnten zeigen, dass die Aktivierung des NF-kappaB-Signalweges über Integrine und Cytokine eine wichtige stimulatorische Wirkung auf die Entstehung und das Fortschreiten entzündlicher Prozesse in den Gefäßen hat. Michael Bader und Mitarbeiter konnten erstmals die Rolle des mas Proto-Oncogens als Rezeptor für Angiotensin 1–7 dokumentieren sowie die kardioprotektive Funktion dieses Angiotensin-II-Metaboliten aufzeigen.

Der Arbeitsgruppe von Thomas Willnow gelang in Zusammenarbeit mit Kollegen der Universität Aarhus der Nachweis, dass Steroidhormone wie Androgene und Östrogene mittels rezeptor-vermittelter Endozytose in Zellen aufgenommen werden können. Diese Befunde widerlegen die gängige Lehrmeinung, dass Steroidhormone ausschließlich über unspezifische, freie Diffusion in Zielgewebe des Körpers gelangen. Die Blockade solcher spezifischer Aufnahmemechanismen für Steroidhormone sind möglicherweise neue therapeutische Ansätze zur Behandlung steroidabhängiger Tumore der Brust und Prostata.

aimed at modulating delivery of steroid hormones to target tissues in patients.

Heart Disease

Cardiac development, myocardial injury and repair, hereditary forms of heart failure and sudden death, and molecular and clinical imaging are the main areas of research interests. Several milestones have been achieved recently. Rüdiger von Harsdorf and Rainer Dietz characterized the role of pro- and anti-apoptotic molecules in the heart, of which ARC seems to have a protective role against circulatory stress. Inhibition of apoptosis and induction of cardiac cell division (not just mitosis!) may, some day, provide therapeutic options to heal infarcted myocardium. The elucidation of new factors essential for cardiac morphogenesis is one focus of Salim Abdelilah-Seyfried's group. The recent characterization of the molecular defects in the *heart and soul (has)* and *nagie oko (nok)* zebrafish mutants provide insights into early phases of cardiac morphogenesis (cardiac tube formation).

Another lesson from embryonic cardiac pathology lead to the discovery of a new cardiomyopathy disease gene in humans. When plakophilin-2 is ablated in the mouse, no regular cell-cell contacts can form and mouse embryos die early as shown by Walter Birchmeier's group. Ludwig Thierfelder and colleagues hypothesized that plakophilin-2 might play a role in patients suffering from a hereditary cardiomyopathy associated with arrhythmias and sudden cardiac death. In a large cohort of these cardiomyopathy patients, a high number of disease causing mutations were discovered and such patients can now be readily identified and receive effective and even prophylactic treatment.

Another translational approach leading to several clinical hypotheses was generated from a basic science lab (Gerd Wallukat's group) when a number of autoantibodies directed against various cell surface receptors were shown to adversely modify the course of patients with heart failure, preeclampsia, or renal graft rejection. Therapeutic removal of such autoantibodies will soon be implemented in clinical practice.

Metabolic Diseases, Genetics, Genomics, and Bioinformatics

Elucidation of the human and other mammalian genomes heralds a new area in biomedical research. Major challenges in the future will be to assign functions to the wealth of sequence information generated in the various genome programs. Thus, high throughput sequence analysis and bioinformatics technologies have to be developed and applied to the positional cloning of disease genes in monogenic and complex traits of metabolic disturbances.

Towards these goals, recent major achievements in this program include the study by Hans Knoblauch and Friedrich C. Luft who, in a collaboration with Peter Nürnberg and Jens Reich, performed a detailed investigation of common lipid-gene variants and lipid levels in 250 German families. The subjects numbered over 1000 and spanned 3 generations. The

Herzerkrankungen

Die Entschlüsselung genetischer Grundlagen der Herzentwicklung, der Schädigung und Regeneration des Myokards, familärer Erkrankungen des Herzversagens sowie die Entwicklung molekularer und klinischer bildgebender Verfahren sind zentrale Forschungsfelder dieses Programmthemas. Eine Vielzahl wichtiger Meilensteine konnten in diesen Forschungsfeldern in den vergangenen 2 Jahren erreicht werden. Rüdiger von Harsdorf, Rainer Dietz und Mitarbeiter haben die Rolle pro- und anti-apoptotischer Faktoren im Myokard untersucht und dabei einen wichtigen Beitrag des Proteins ARC als protektivem Faktor vaskulärer Stressbedingungen identifiziert. Zukünftig könnte die Intervention bei pro-apoptotischen zellulären Vorgängen ein wichtiges Instrument zur Therapie des geschädigten Herzmuskelgewebes darstellen.

Die Aufklärung neuer genetischer Signalwege in der Herzentwicklung ist Schwerpunkt der Arbeiten von Salim Abdelilah-Seyfried. Seiner Gruppe gelang in neueren Studien die Beschreibung der molekularen Defekte in den Zebrafischmutanten *heart and soul (has)* und *nagie oko (nok)*, welche wichtige neue Einblicke in die frühe Phase der Herzmorphogenese geliefert haben.

Neue Erkenntnisse aus dem Studium kardialer Entwicklungsdefekte in Tiermodellen führten ebenfalls zur Aufklärung der molekularen Ursachen häufiger Kardiomyopathien beim Patienten. Wie die Gruppe von Walter Birchmeier zeigen konnte, bedingt die Inaktivierung des Plakophilin-2-Gens in Mausmodellen den Verlust der Zell-Zell-Interaktionen im embryonalen Herzen und frühe Lethalität. Ausgehend von diesen Befunden gelang es Ludwig Thierfelder und Mitarbeitern, vergleichbare genetische Defekte im Plakophilin-2-Gen beim Patienten als Ursache einer häufigen Form familiärer Kardiomyopathie (mit Arrhythmie und plötzlichem Herztod) nachzuweisen. Basierend auf diesen Befunden lassen sich betroffene Patienten jetzt frühzeitig diagnostisch erfassen und mit geeigneten interventionellen und prophylaktischen Maßnahmen behandeln.

Ein anderer translationaler Forschungsansatz der Gruppe um Gerd Wallukat führte zur Aufklärung der zentralen Rolle von Autoantikörpern gegen verschiedene Oberflächenrezeptoren als Auslöser pathologischer Prozesse bei Patienten mit Herzversagen, Präeklampsie und Nierentransplantatabstoßung. Therapeutische Maßnahmen zur Entfernung solcher Autoantikörper aus dem Serum Betroffener mittels Plasmapherese werden zukünftig Eingang in die klinische Praxis finden.

Metabolische Erkrankungen, Genetik, Genomik und Bioinformatik

Die Entschlüsselung des Erbguts des Menschen und das anderer Säuger ist ein Meilenstein der modernen biomedizinischen Forschung. Noch größer jedoch als die Herausforderung der Entschlüsselung des menschlichen Erbguts ist die Aufgabe, die gewonnene genetische Information auszuwerten und funktionell zu charakterisieren. Ein wesentliches Ziel des Forschungsschwerpunktes Genetik, Genomik und Bioinformatik besteht darin, neue molekulargenetische, bioinformatische

group performed a comprehensive SNP analysis in 13 genes. The relative effects of the individual genes on the lipid phenotypes were elucidated in their study. Their work ushers in the 500K SNP analyses that are on the horizon.

The group of Norbert Hübner has focused on a novel approach of integrating genome-wide expression profiling with linkage to identify genes underlying complex traits. The researchers applied this approach to the regulation of gene expression in rat recombinant inbred strains, a leading resource for genetic analysis of the metabolic syndrome. In two tissues important to the pathogenesis of this syndrome, they mapped cis- and trans-regulatory control elements for expression of many genes across the genome. This data set lead to new insights into genes and regulatory pathways underlying the extensive range of metabolic and cardiovascular disease phenotypes that segregate in these recombinant inbred strains.

Previous studies in animal models suggested an important contribution of infiltrating monocytes in adipose tissues to the pathological alteration in metabolism during adipositas. Using microdialysis experiments, Jens Jordan and co-workers now have confirmed this central mechanism in human subjects.

und biostatistische Verfahren zu entwickeln, um die Flut genetischer Informationen analysieren zu können und für funktionelle Untersuchungen bei der Kartierung monogener und komplexer genetischer Ursachen von Stoffwechselstörungen einzusetzen.

Im Rahmen ihrer Studien haben Hans Knoblauch und Friedrich C. Luft in Zusammenarbeit mit Peter Nürnberg und Jens Reich eine detaillierte Kartierung häufiger genetischer Varianten in Genen des Lipidstoffwechsels durchgeführt und deren Einfluss auf die Höhe individueller Plasmalipidspiegel in über 250 Familien bestimmt.

Die Arbeitsgruppe von Norbert Hübner hat sich auf die Aufklärung komplexer genetischer kardiovaskulärer Erkrankungen mittels Genom-weiter Expressionsanalyse konzentriert. Die Wissenschaftler haben diese Methodik eingesetzt, um Genexpressionsprofile in Ratten-Inzuchtstämmen metabolischer Störungen zu erstellen und cis- und trans-regulatorische Elemente zu identifizieren, welche eine Vielzahl metabolischer und kardiovaskulärer Parameter im Säugerorganismus bestimmen.

Ausgehend von Untersuchungen in Tiermodellen, die nahelegen, dass in Fettgewebe eingewanderte Monozyten wesentlich zu pathologischen Veränderungen bei Adipositas beitragen, haben Jens Jordan und Mitarbeiter diese Hypothese mittels Microdialyse-Untersuchungen im Patienten geprüft und validiert.

Molecular Cardiovascular Research

Thomas E. Willnow

Introduction

The low-density lipoprotein (LDL) receptor is a 150 kDa endocytic receptor that mediates the cellular uptake of lipoprotein particles and plays a central role in the removal of lipids from the systemic circulation. In patients with a genetic defect of the LDL receptor (Familial Hypercholesterolemia), a massive increase in the concentration of circulating plasma lipoproteins results in hyperlipidemia and, consequently, in atherosclerosis and coronary artery disease. In recent years, a number of novel receptors have been identified that are structurally related to the LDL receptor and that are designated members of the LDL receptor gene family. Given the central role of the LDL receptor in the cardiovascular system, equally important roles for other receptors in this gene family are anticipated. The focus of our studies is the elucidation of the functions that LDL receptor-related receptors (LRPs) play in the (patho)physiology of the cardiovascular system, particularly in the systemic and cellular lipid metabolism. Towards this goal, we are using gene-targeting approaches to generate mouse models with ubiquitous or conditional LRP deficiencies and to analyze the consequences of the receptor gene defects in vivo. In the past two years, we have identified important new functions of lipoprotein receptors in the development of the central nervous system and the reproductive organs, and the molecular mechanisms underlying human diseases in these areas.

Role of lipoprotein receptors in forebrain development

Megalin is an LRP expressed in the neuroepithelium and the yolk sac of the early embryo. The receptor acts as multi-ligand scavenging receptor mediating the cellular uptake of lipoproteins and lipid/carrier complexes. Loss of megalin expression in knockout mice results in forebrain defects and in holoprosencephaly (HPE), indicating an essential yet unidentified function in brain development. HPE is defined as a failure of the prosencephalon to separate along the mid-sagital axis into discrete hemispheres. This defect is likely to be the result of defective patterning during development involving improper specification of the rostral portion of the neural tube. HPE is the most common brain anomaly of the human embryo and affects 1 in 250 pregnancies. Disturbances in cholesterol metabolism seem to play a causal role in this disease as indicated by the holoprosencephalic defects seen in patients suffering from Smith-Lemli-Opitz Syndrome, a heritable defect of cholesterol biosynthesis. We have used mice with conditional megalin gene inactivation in the yolk sac and/or the embryo proper to uncover the role of the receptor in forebrain formation. We demonstrated that expression of megalin in the neuroepithelium but not in the yolk sac is required for brain development. In the neuroepithelium, megalin deficiency causes defects in dorso-ventral patterning of the telencephalon including loss of expression of sonic hedgehog (Shh) in the ventral (figure 1) and enhanced expression of bone morphogenic protein 4 (Bmp4) in the dorsal neural tube. These findings indicate a crucial role for the lipoprotein receptor megalin in the regulation of the activity of SHH and BMP4, two morphogens that act as central regulators of neural tube patterning.

Endocytic receptors for steroid hormones

According to current concepts, steroid hormones enter target cell by free diffusion through the plasma membrane. However, we have shown previously that some tissues use endocytosis to actively internalize the steroid hormone 25-OH vitamin D3 bound to carrier proteins, thus acquiring large amounts of this important regulator independent of diffusion processes. Initially considered a unique feature of vitamin D metabolites, we now have proof that endocytosis is a general concept that also applies to other steroid hormones such as androgens and estrogens. We demonstrated that megalin, an endocytic receptor for lipid/carrier complexes, is expressed in the principal cells of the epididymis and in the uterus, tissues that require large amounts of sex steroids for normal function. We further showed that megalin mediates the cellular uptake of androgens and estrogens bound to the carrier sex hormone-

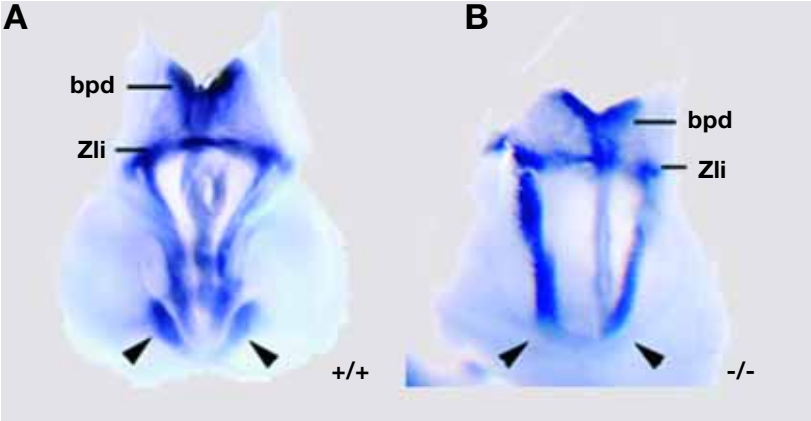


Figure 1 *Shh* expression in wild type (A) and megalin$^{-/-}$ (B) embryos.
In situ hybridization of frontal head aspects of wild type (A) and *megalin*$^{-/-}$ E10.5 embryos (B) depicting *Shh* expression in the rostral ventral neural tube (arrowheads) of wild type but not knockout animals. Expression of *Shh* in other areas of the forebrain such as in the basal plate of the diencephalon (bpd) or the zona limitans intrathalamica (Zli) is identical in both genotypes.

binding globulin (SHBG) (figure 2). Finally, we uncovered that megalin deficiency in mice results in impaired descent of the testes into the scrotum in males and in blockade of vagina opening in females, two processes that are critically dependent on sex steroid signaling. Similar defects are seen in rodents treated with androgen or estrogen receptors antagonists, indicating insensitivity to steroid hormones as the underlying molecular defect in megalin deficient mice. Our findings demonstrate a direct link between endocytosis of steroid hormones and sex steroid action in vivo, and they provide further evidence for a paradigm shift in our concept of the metabolism and action of steroid hormones.

SorLA, a neuronal lipoprotein receptor that regulates processing of the amyloid precursor protein

Recently, a novel receptor, SorLA-1, was uncovered that combines motifs of the LDL receptor gene family with structural elements found in the yeast vacuolar sorting receptor Vps10p. Although, SorLA has been shown to bind ligands relevant for lipoprotein metabolism, such as apolipoprotein E, the physiological function of this receptor was unclear. However, its homology to sorting receptors that shuttle between plasma membrane, endosomes, and Golgi suggested a related function in neuronal trafficking processes. Because expression of SorLA is reduced in the brain of patients with Alzheimer's disease (AD), we tested the involvement of this receptor in intracellular transport and processing of the amyloid precursor protein (APP) to the amyloid β-peptide (Aβ), the principal component of senile plaques. APP follows a complex trafficking pathway that determines alternative processing into the soluble sAPP· fragment (non-amyloidogenic pathway) or into the Aβ peptide (amyloidogenic pathway). The cellular mechanisms that direct APP into either of the two processing pathways remain elusive. Here, we demonstrated that SorLA interacts with APP in vitro and in living cells (figure 3), and that both proteins co-localize in endosomal and Golgi compartments. Overexpression of SorLA in neurons causes redistribution of APP to the Golgi and decreased processing to Aβ whereas ablation of SorLA expression in knockout mice results in increased levels of Aβ in the brain similar to the situation in patients with AD. Thus, SorLA acts as a sorting receptor that protects APP from processing into Aβ and thereby reduces the burden of amyloidogenic peptide formation. Consequently, reduced receptor expression in human brain may increase Aβ production and senile plaque formation, and promote spontaneous AD.

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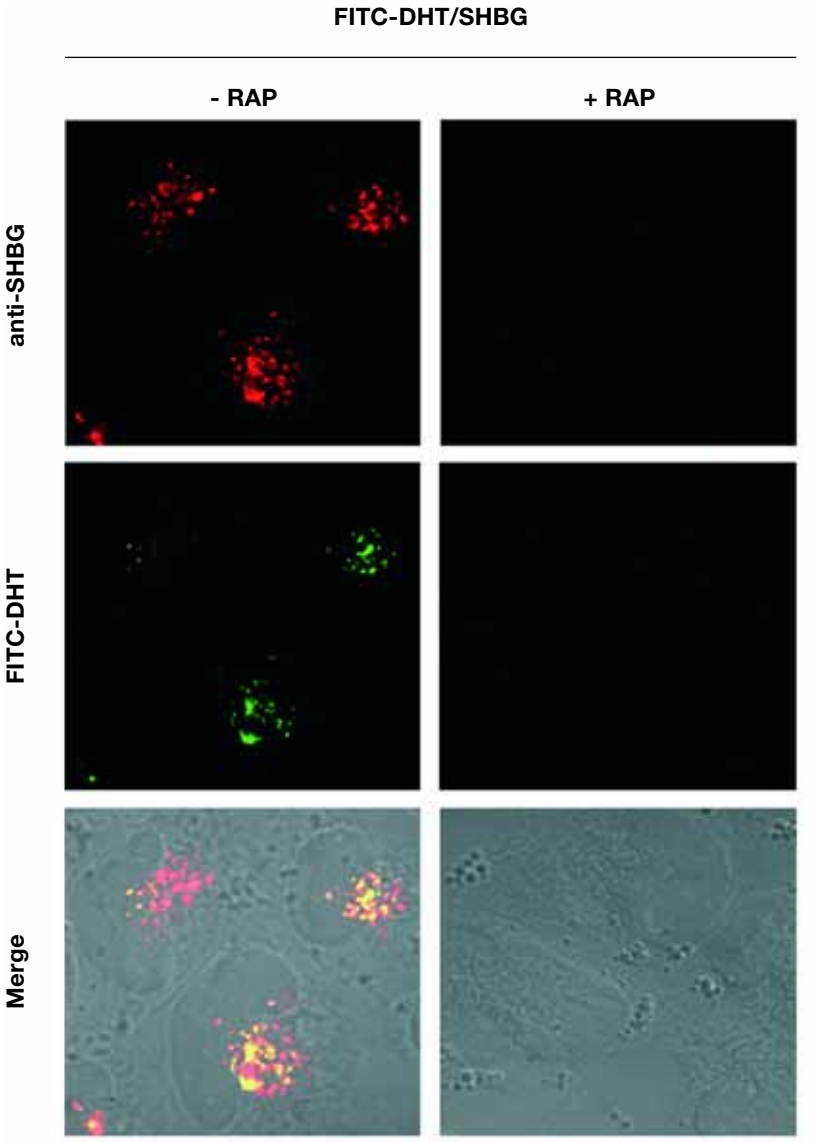

Figure 2
Endocytic uptake of dihydrotestosterone/SHBG complexes in cells expressing megalin.
Cells were incubated with preformed complexes of FITC-labeled dihydrotestosterone(DHT)/SHBG in the presence or absence of RAP, an inhibitor of ligand binding to megalin. Subsequently, the cellular uptake of FITC-DHT and SHBG (anti-SHBG antibody, followed by secondary alexa 660-labeled IgG) were detected by confocal immunofluorescence microscopy. Endocytic uptake of androgen/carrier complexes can be seen in cells with megalin activity (-RAP) but not in cells treated with receptor antagonists (+RAP).

Patent Applications

1. *Patent application US pat. Appl. No. 10/131,597:*
   Agents for prevention of organ damage induced by therapeutic agents
2. *Patent application PCT/IB02/01393:*
   Agents for prevention of organ damage induced by therapeutic agents
3. *Patent application PA 2003 00459:*
   Use of compounds for the prevention of drug-induced cell toxicity.
4. *Patent application PCT/DK03/00919:*
   Modulation of activity of neurotrophins

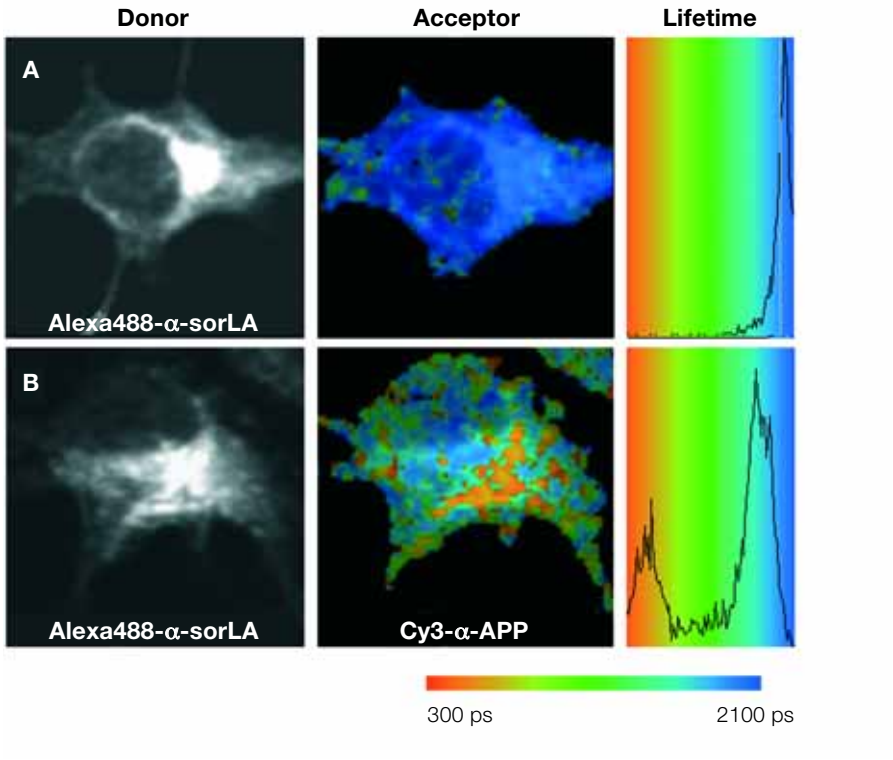

Figure 3
Fluorescence lifetime imaging microscopy (FLIM) of APP and SorLA interaction in N2A cells.
The figure displays intensity images of SorLA immunocytochemistry using donor fluorophore Alexa488-conjugated antibodies (left), and pseudo coloured FLIM images (middle) and lifetime histograms (right), indicating shortening of lifetime from blue to orange/red color in the absence (A) or presence (B) of acceptor fluorophore Cy3. (A) Cells stained for the amino terminal domain of SorLA with donor fluorophore Alexa488 in the absence of acceptor (lifetime 2007 ± 12 psec). (B) Cells stained for SorLA with donor fluorophore Alexa488 in the presence of acceptor Cy3 on the ectodomain of APP. The shortening of the fluorescence lifetime on SorLA to 1365 ± 139 psec indicates a close physical association with the acceptor on APP.

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Molecular Biology of Peptide Hormones

Michael Bader

The group is interested in the molecular biology and function of hormone systems involved in cardiovascular regulation. Besides the cloning and characterization of genes for the components, the physiological functions of the systems are analyzed by the production and analysis of transgenic and gene-targeted animal models.

Renin-angiotensin system

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, and kidney, are involved in these processes. Therefore, transgenic rats with local up- or downregulation of RAS components in brain, heart, and vessels (e.g. by the organ-specific expression of antisense-RNA or of a peptide-liberating protein) were produced. Using these rats, we could show that central angiotensin modulates circadian blood pressure rhythms, the baroreceptor reflex and, as a non-cardiovascular parameter, alcohol consumption. Furthermore, it is involved in the hypertensive and hypertrophic effects of circulating angiotensin. Other genetically altered mouse and rat models for non-classical RAS components, such as ACE2, the renin receptor, the mas proto-oncogene, and angiotensin(1-7), have elucidated the physiological function of these factors.

Kallikrein-kinin system

The kallikrein-kinin system (KKS) is an important hormone system for cardiovascular regulation 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. Furthermore, the analysis of transgenic rats overexpressing B1 receptors in vessels and of knockout mice lacking one or both kinin receptors showed that the KKS is essential for the hypotension occurring after septic shock.

Natriuretic peptide system

There are 3 natriuretic peptides, ANP, BNP, and CNP, which interact with two receptors, NPR-A and NPR-B, to induce a multitude of actions in heart, kidney, and vessels. 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.

Serotonin system

Serotonin is at the same time a very important neurotransmitter in the brain and a major factor released by platelets in the circulation. After our discovery of the second serotonin synthesizing enzyme, tryptophan hydroxylase 2 (TPH2), which is responsible for serotonin synthesis in the central nervous system, we could show that this gene shows linkage to psychiatric diseases. Mice deficient in the peripheral enzyme, TPH1, exhibited defects in platelet function due to a blunted release of α-granules containing von Willebrand factor at sites of vessel injury. Serotonin stimulates the release of α-granules by a novel signalling pathway, the serotonylation of small GTPases. Unexpectedly, TPH1-deficient animals have a completely normal gut function despite a very high activity of TPH1 in this organ of normal mice. However, homeostasis of the mammary gland is severely impaired by the lack of TPH1.

Transgenic and stem cell technology

The group is also interested in developing transgenic and stem cell technology in the mouse and even more importantly in the rat. In order to detect crucial molecules for the development of the differentiation of serotonergic neurons, mouse embryonic stem (ES) cells are genetically modified and selected during in vitro differentiation to enrich for serotonergic precursors. In order to allow targeted genetic alterations in the rat, several techniques are being employed. Rat ES cells were isolated but did not allow germline transmission. Therefore, nuclear transfer technologies have been developed and optimized for the rat. Furthermore, transgenic rats are produced carrying constructs which express small interference RNAs suited to downregulate specific genes.

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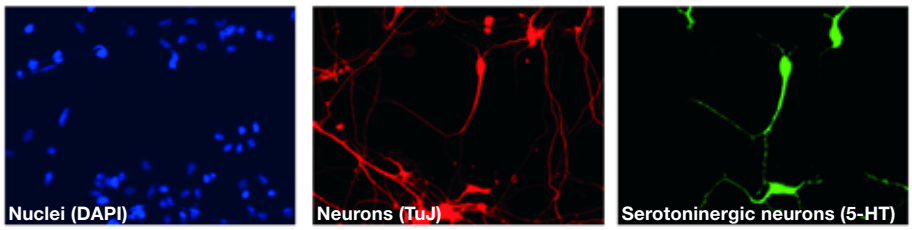

Figure 1
Serotonergic neurons developed *in-vitro* from mouse embryonic stem cells.
The same *in-vitro* differentiated embryonic stem cells were stained for nuclei (DAPI) to show all cells, for tubulin β-III (TuJ) to mark neurons and for serotonin (5-HT) to highlight serotonergic neurons.

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Hypertension, Vascular Disease, Genetics, and Nephrology

Friedrich C. Luft

Summary

The group is interested in molecular and genetic mechanisms contributing to blood pressure regulation, cardiovascular, and renal diseases. Ralph Kettritz is pursuing mechanisms responsible for proteinase-3 (PR3) and myeloperoxidase (MPO) antibody-induced vasculitis. The neutrophil is the key cell in these diseases. He elucidated novel signaling pathways, genetic control of PR3 cell surface expression, and successfully studied neutrophil differentiation and PR3 expression from stem cells. Volkmar Gross pursued long-term telemetric physiological studies of gene-disrupted mice. Hans Knoblauch performed a detailed SNP analysis of 13 lipid-relevant genes in 250 families. Atakan Aydin elucidated the SNPs in long-QT syndrome genes. Sylvia Bähring and Martin Kann pursued the role of inversions, deletions, and reinsertion rearrangements in families with autosomal-dominant hypertension and brachydactyly.

Vasculitis

Systemic vasculitis (inflammation of medium to small blood vessels) is caused to a large extent by antibodies directed against neutrophil components, notably against proteinase-3 (PR3) and myeloperoxidase (MPO). A characteristic immunofluorescent staining pattern termed anticytoplasmic neutrophil antibodies (C-ANCA) is seen with the former (Wegener's granulomatosis), while a perinuclear staining pattern termed perinuclear anticytoplasmic neutrophil antibodies (P-ANCA) is observed with the latter. ANCA-induced vasculitis is a common cause of end-stage renal failure, upper airway disease, and pulmonary disease. Ralph Kettritz and his group focused on neutrophil signaling. They found that integrins and cytokines activate nuclear transcription factor-kappaB in human neutrophils. They showed that beta(2) integrins provide costimulatory signals allowing soluble mediators to activate the NF-kappaB pathway when the cells are fixed to matrix even when they are not capable of doing so when the cells are in suspension. This effect may become important when human neutrophils leave the circulating blood and migrate through extracellular matrix during inflammation. In other studies, the group showed that human hematopoietic stem cells could be cultivated into human neutrophils that express PR3 on their surface (figure). This membrane PR3 expression is strongly influenced by genetic variance and is of great clinical importance to the disease process. Finally, Ralph Kettritz is busy investigating the effect of fever-like temperatures on neutrophil signaling. Fever-like temperatures protect circulating neutrophils from TNF-alpha-mediated apoptosis, but not the adherent cells. The findings support the notion that fever is a parsimonious protective response.

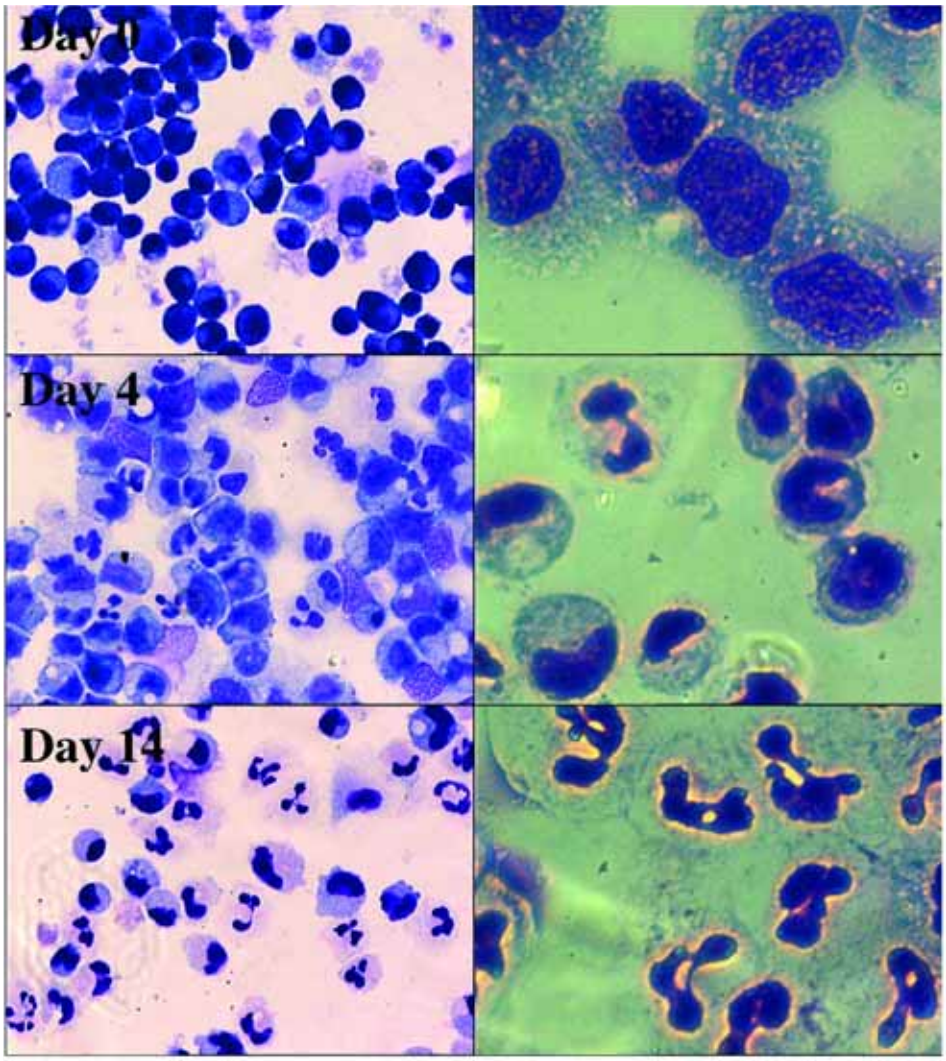
Systems biology in mice

Volkmar Goss and Michael Obst have continued to perfect sophisticated, long-term, telemetric cardiovascular studies in gene-modified mice. The group showed increased systemic blood pressure and enhanced vascular smooth muscle contractility in TRPC6-/- mice. They found that mice deficient in the regulator of G protein signaling (RGS2) have hypertension, increased sympathetic tone, and baroreflex resetting. They investigated renal function and concentrating ability of mice deficient in uromodulin. The group tracked cardiac output, peripheral vascular resistance, and systemic blood pressure in two models of evolving hypertension, L-NAME and DOCA-Salt. They found that contrary to what was previously believed, both models generate increased peripheral vascular resistance rather than increased cardiac output during the development of hypertension. Finally, in a closely coordinated "Of Mice and Men" study, the group investigated spontaneous baroreflex sensitivity in conscious mice through parasympathetic activation. Parallel studies were performed in human subjects. Both studies were published back-to-back and gave similar insight into central regulation of parasympathetic tome via adrenergic alpha2 receptor activation. The conscious murine 600-bpm heart rate and 100-breaths/min respiratory rate were challenges that had to be surmounted.

Molecular genetics

Hans Knoblauch and Friedrich C. Luft (Franz Volhard Clinic) collaborated with Peter Nürnberg and Jens Reich (MDC) and performed a detailed investigation of common lipid-gene variants and lipid levels in 250 German families. The subjects numbered over 1000 and their ages spanned 3 generations. The group performed a comprehensive SNP analysis in 13 genes. The relative effects of the individual genes on the lipid phenotypes were elucidated in their study. Their work ushers in the 500K SNP analyses that are on the horizon.

Atakan Aydin identified SNPs in the genes responsible for the long-QT syndrome, a condition causing sudden cardiac death. He found 35 SNPs, 10 of which were not previously known. Ten SNPs were in KCNE1, six in HERG, eight in KCNQ1, four in KCNE2, and seven in SCN5A. Four SNPs were associated with QTc interval in his subjects, one in KCNE1, one in KCNE2, and two in SCN5A. Two of these SNPs had also not previously been described. Atakan Aydin patented two new methods for rapid SNP identification and genotyping. His PhD thesis was judged *summa cum laude*.



Differentiation of CD34+ hematopoietic stem cells with G-CSF. Expanded CD34+ cells at day 0 and after 4 and 14 d of treatment with G-CSF were cytospun and stained with Wright-Giemsa. Microscopy indicates the progressive appearance of cells with morphologic signs of neutrophilic maturation. A typical of two independent experiments is depicted. Magnification, x40 in left images; x100 in right images.

Sylvia Bähring, Martin Kann, Atakan Aydin, and Friedrich C. Luft have directed their energies on elucidating autosomal-dominant hypertension and brachydactyly, a condition that they mapped earlier to the short arm of chromosome 12. The group showed recently that the problem resides in a complex chromosomal rearrangement on chromosome 12p instead of a mutated gene within the linkage interval. Additional complex phenotyping in the Clinical Research Center of the Franz Volhard Clinic showed that three candidate genes in the interval, PDE3A, Kir6.1, and SUR2, all functioned normally and were therefore declared innocent. Very recently, Martin Kann performed a detailed 24-clone BAC contig interphase FISH investigation of four families and one isolated patient with the syndrome. He found that inversions of the centromeric part of the locus were present in all affected persons; they were unique for any given family. However, the inversions lacked a common breakpoint and differed greatly in size. In one instance, the inversion even extended beyond the boundaries of the previously mapped linkage interval. None of the inversions led to disruption of any known coding sequence. Since no known genes are affected by the mutations, possible causes for the syndrome include as yet unknown genes or non-coding RNA. A position effect must also be considered that could alter the interplay between a regulatory element and its respective gene. We are currently investigating these possibilities.

Hypertension-induced vasculopathy

This topic has become the responsibility of Dominik N. Müller (see Helmholtz Fellows). Anette Fiebeler has pursued the roll of mineralocorticoid receptor signaling in vascular injury. Several important papers have emanated from her work. Anette Fiebeler recently achieved faculty rank through her research on the role of the mineralocorticoid receptor in the heart and elsewhere as a signaling operator for remodeling events.

Milestones

This group, the group led by Jens Jordan, and the Helmholtz Fellows Dominik N. Müller, Maik Gollasch, and Matthias Köhler, constitute the research section of the Department of Nephrology and Hypertension, Medical Faculty of the Charité, Campus-Buch, chaired by Friedrich C. Luft. Jens Jordan and Ralph Kettritz were appointed Professors of Medicine, Campus-Buch in 2004. Maik Gollasch, who had served as Associate Professor of Physiology, Louisiana State University, New Orleans, Louisiana, USA, was appointed as Professor of Medicine, Campus-Virchow Klinikum in 2005. Adrian Schreiber, Kai Schmidt-Ott, and Roland Schmitt are currently in the USA pursuing DFG-sponsored research fellowships. The latter two are recipients of the prestigious Emmy Noether Award.

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Cytochrome P450-dependent Eicosanoids in the Regulation of Cellular and Organ Function

Wolf-Hagen Schunck

Summary

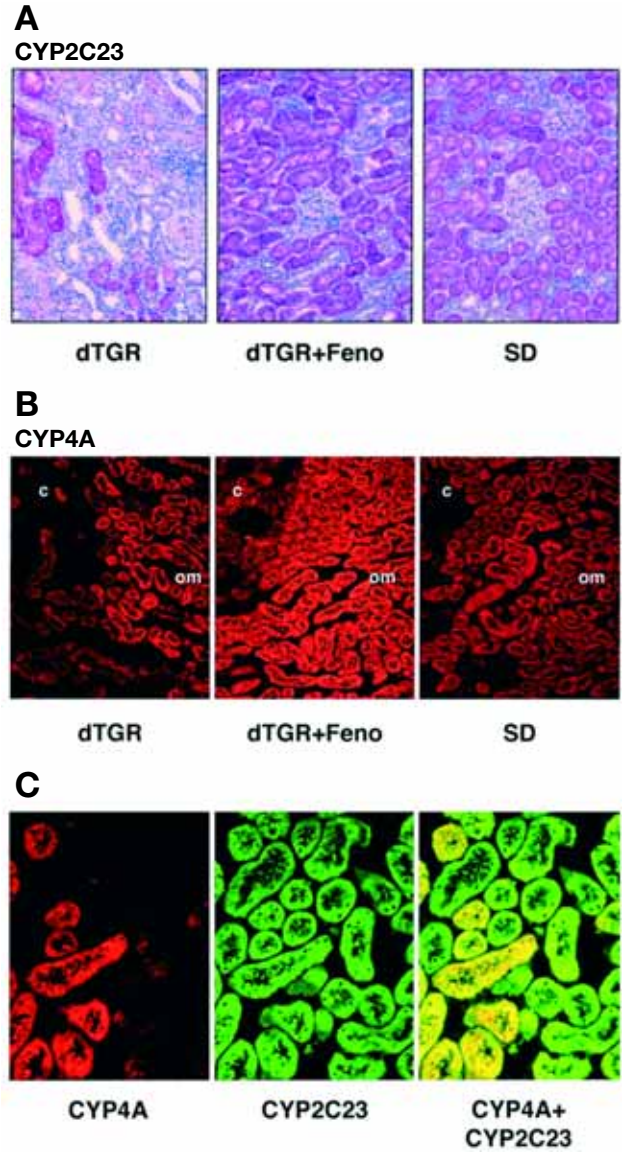
The laboratory is interested in the role of arachidonic acid (AA) metabolizing cytochrome P450 (CYP) enzymes in the regulation of vascular tone, renal function, and the control of inflammation. In collaboration with other groups at the MDC and the Franz Volhard Clinic, we have examined pathophysiological changes in renal CYP-dependent AA metabolism using mice and rat models of hypertension and end-organ damage. Our previous studies revealed that angiotensin II (Ang II)-induced hypertension is associated with a down-regulation of CYP enzymes required for the production of epoxyeicosatrienoic acids (EETs) in the kidney. Since EETs are mediators in the action of vasodilatory hormones, have anti-inflammatory properties, and promote salt excretion, these findings led to the hypothesis that a shortage of these eicosanoids may contribute to the development of high blood pressure and renal damage.

Cardiovascular disease

We have focused on PPAR-alpha activation, CYP-dependent AA metabolism, and renal damage. Recently, we addressed the question whether hypertension and target organ damage can be ameliorated by treatments that improve the expression and activity of EET-producing CYP-enzymes. We found that an activator of the peroxisome proliferator-activated receptor alpha (PPAR-alpha) strongly induced renal CYP-dependent EET generation and protected from Ang II-induced hypertension and renal injury. These experiments were performed using double transgenic rats (dTGR) that overexpress both the human renin and angiotensinogen genes. Untreated dTGR developed severe hypertension and died of cardiac and renal damage. Treatment with the PPAR-alpha activator fenofibrate reduced mortality to zero and normalized blood pressure and albuminuria. Fenofibrate strongly induced renal microsomal AA-epoxygenase activities and the expression of the major CYP-isoform responsible in the rat kidney for EET production (CYP2C23). In the course of these studies, we found a novel catalytic capacity of the CYP2C23 enzyme. CYP2C23 is highly efficient in epoxidizing 20-hydroxyeicosatetraenoic acid (20-HETE). The main product was identified as 20-hydroxy, 8,9-epoxyeicosatrienoic acid (HEET). HEETs are endogenous high-affinity PPAR-alpha ligands. Therefore, we speculate that fenofibrate triggered a positive feed back mechanism that involved PPAR-alpha activation, CYP2C23 induction, production of EETs and HEETs which then enhanced PPAR-alpha activation. We have also focused on omega-3 polyunsaturated fatty acids (n-3 PUFAs), CYP-dependent metabolism, and beneficial cardiovascular effects. Numerous studies have demonstrated that diets rich in n-3 PUFAs, such as fish oil, have beneficial cardiovascular effects. We demonstrated that treatment of dTGR with n-3 PUFAs protected against Ang II-induced end-organ damage. This protection may have involved an anti-inflammatory action of the n-3 PUFAs since we found a down-regulation of the proinflammatory transcription factors AP-1 and NF-kappaB. However, the molecular mechanisms of n-3 PUFA action are largely unknown and probably complex. We speculated that alterations in CYP-dependent eicosanoid production could contribute to the vasodilatory and anti-inflammatory effects of dietary n-3 PUFAs. As a first step to test this hypothesis, we studied the ability of AA-metabolizing CYP-isoforms to accept eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the main n-3 PUFAs contained in fish oil, as alternative substrates. Our results demonstrate that both EET- and 20-HETE-producing isoforms of human, rat, and mouse convert EPA and DHA with high catalytic efficiencies. CYP4A and CYP4F enzymes generated predominantly 19-/ 20-OH-EPA and 21-/22-OH-DHA whereas CYP2C and CYP2J enzymes produced different regio- and stereoisomeric epoxides of EPA and DHA. Interestingly, the n-3 double bond was a preferred site of epoxidation. The resulting metabolites (17,18-epoxy-EPA and 19,20-epoxy-DHA) are unique in having no homologs in the series of AA metabolites. 17,18-epoxy-EPA showed the capacity to dilate rat renal microvessels substantiating our previous finding that this metabolite is a strong activator of calcium-dependent potassium channels in vascular smooth muscle cells. Taken together, these studies show that n-3 PUFAs have the capacity to compete with AA for the conversion by CYP enzymes and the generation of biologically active eicosanoids. Based on these results, we are now interested in the signal transduction pathways influenced by CYP-dependent n-3 PUFA metabolites in endothelial cells, vascular smooth muscle cells, and cardiac myocytes.

Genetics of CYP polymorphisms and CYP enzyme metabolic function

In collaboration with the Department of Clinical Pharmacology at Charité Midtown Campus, we have studied the hydroxylation activity of common allelic human CYP1A1 variants. We found that the CYP1A1.2 (Ile(462)Val) variant exhibits superior activity because of a higher V(max). This observation is highly relevant to the risk of estrogen-induced cancers and also to cardiovascular disease. We have also expressed CYP1A1 and human NADPH-cytochrome P450 reductase in *Spodoptera frugiperda* insect cells. With the help of this system, we found that the capacity of human CYP1A1 to metabolize AA and EPA greatly affects the production of



CYP2C23 **(A)** and CYP4A immunoreactivity **(B)** in renal cortical tubules of untreated double transgenic rats (dTGR), fenofibrate treated dTGR (dTGR+Feno) and Sprague-Dawley control rats (SD). Fenofibrate, a PPAR-alpha agonist, restores CYP2C23 activity and induces CYP4A protein in this model of angiotensin II-induced target organ damage. **(C)**: CYP4A (red) and CYP2C23 (green) are co-localized in several cortical tubules (orange in the merged picture). The enzymes cooperate to produce 20-hydroxy,8,9-epoxyeicosatrienoic acid (HEET). HEETs are endogenous high-affinity PPAR-alpha ligands.

active metabolites. The relevance of these observations has to do with inducing the enzyme by polycyclic aromatic hydrocarbons.

Selected Publications

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Smooth Muscle Cell Electrophysiology, Ion Channel, and Transporter Function

Maik Gollasch
(Helmholtz Fellow)

Summary

The work in the laboratory focuses on the ionic mechanisms responsible for the onset and maintenance of myogenic vascular tone in small arteries. A second area of research is directed at identifying a factor produced by perivascular fat as a modulator of arterial tone. We termed this factor the advential-derived relaxing factor. A third area of area of research focuses on the dicarboxylate transporter encoded by the life-extending gene family Indy (I'm not dead yet).

Calcium sparks and control of myogenic tone

We used gene knockout animals and were successful in showing that the large conductance calcium-activated potassium (BK) channel represents the molecular target of calcium sparks to reduce myogenic tone. Deletion of the pore-forming BK alpha subunit disrupted coupling between calcium sparks and BK channels, leading to increased arterial tone, hyperaldosteronism, and an increase in systemic blood pressure in mice. We also found that the canonical transient receptor potential channel 6 (TRPC6) is important to the control of vascular smooth muscle tone. We showed that constitutively active TRPC3 type channels are upregulated in TRPC6-gene deficient smooth muscle cells, leading to an increase in contractile response. The TRPC6 gene-deficient mice also have systemic hypertension. We are currently examining the role of Cav1.2 L-type Ca^{2+} channels in triggering the release of calcium sparks in arterial smooth muscle cells using smooth muscle cell-specific inactivation of the $Ca_v1.2$ L-type Ca^{2+} channel gene in mice. These mice are termed SMAKO mice. They appear to have a malfunction of the calcium spark/BK channel pathway which, together with TRPC6 type channels, could contribute to hypertension. We are hopeful that these basic research directions will lead to similar hypotheses being tested in humans.

Control of arterial tone by perivascular fat

Virtually all blood vessels are surrounded by variable amounts of adipose tissue. We established the concept of the paracrine role for periadventitial adipose tissue in the regulation of arterial tone, an advential-derived relaxing factor (ADRF), in earlier reports. We showed that perivascular adipose tissue induces vasorelaxation by activating smooth muscle voltage-dependent K_v channels. In collaboration with Dr. W.-H. Schunck's group, we are investigating the effects of P450-dependent epoxygenation of eicosapentaenoic acid on potassium channels. We have initiated a major effort to isolate this material using various purification methods. We believe that perturbations in ADRF release may provide the connection between obesity and arterial hypertension.

Transport properties of INDY

Felix Knauf joined the group as a medical student. He had completed a two-year research fellowship at Yale University with Dr. Peter Aronson, a noted nephrologist and transporter physiologist. Felix needed a few crucial transport experiments to complete his paper. We established the *Xenopus* oocyte system in the laboratory and relied on electrophysiology techniques that were available in the laboratory. Felix was assisted by Nilufar Mohebbi, a nephrologist from the clinic. The study Felix was able to complete here in our MDC laboratory is cited in the report and includes his U.S. collaborators. However, the studies elucidating *Indy* were expanded and continued. Using two electrode voltage clamp and flux measurements in Xenopus oocytes, we were able to show that the life-extending gene *Indy* encodes an exchanger for Krebs-cycle intermediates. We propose that the effect of decreasing *INDY* activity, as in long-lived Indy *Drosophila* mutants, may be to alter energy metabolism in a manner that favors life span extension. This work is currently under review.

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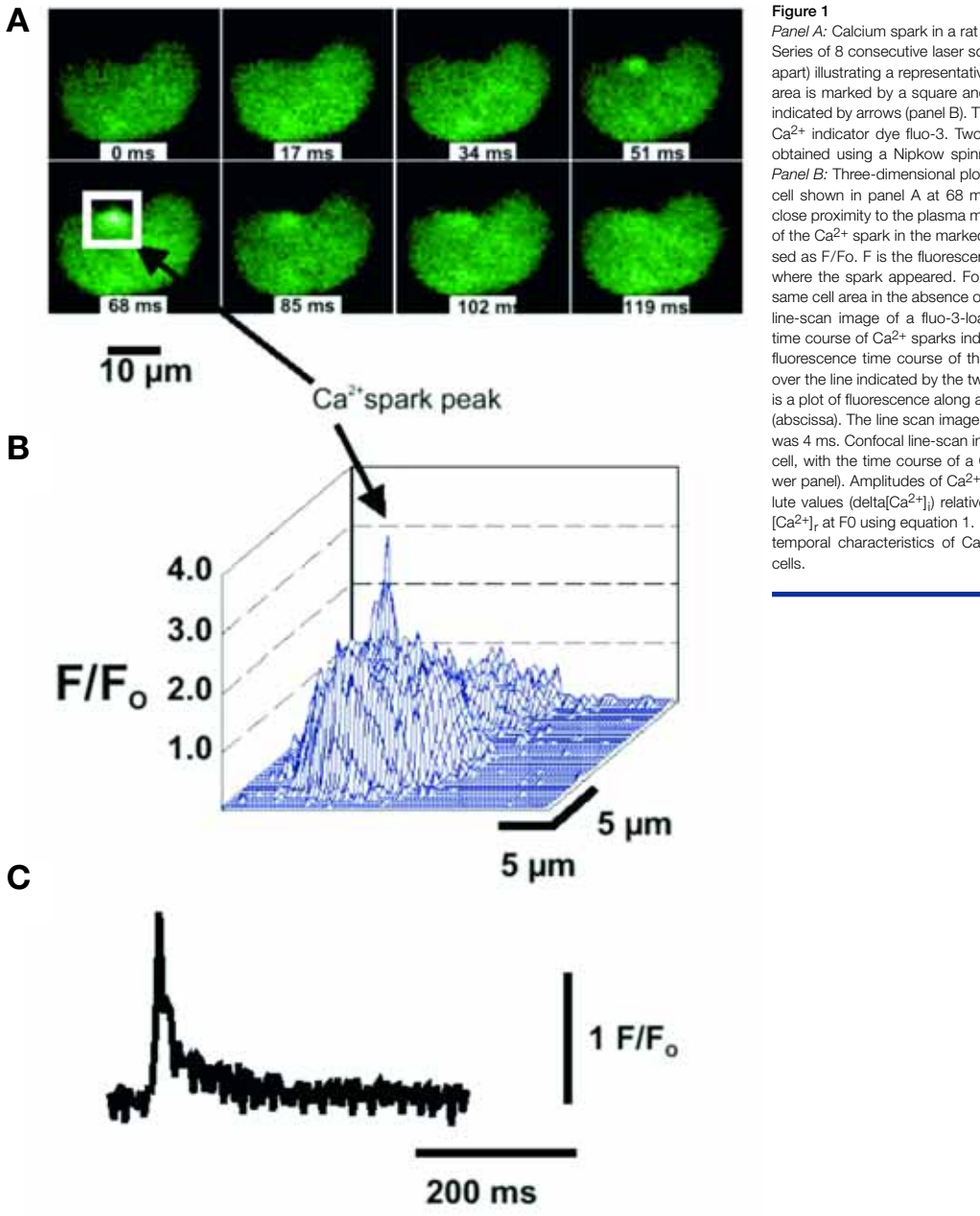

Figure 1

Panel A: Calcium spark in a rat tibial artery smooth muscle cell. Series of 8 consecutive laser scanning confocal images (17 ms apart) illustrating a representative Ca2+ spark. The Ca^{2+} spark area is marked by a square and the peak of the Ca^{2+} spark is indicated by arrows (panel B). The myocyte was loaded with the Ca^{2+} indicator dye fluo-3. Two-dimensional (2D) images were obtained using a Nipkow spinning disk confocal microscope. *Panel B:* Three-dimensional plot of fluorescence intensity of the cell shown in panel A at 68 ms. The Ca^{2+} spark occurred in close proximity to the plasma membrane. *Panel C:* Time course of the Ca^{2+} spark in the marked area. The amplitude is expressed as F/Fo. F is the fluorescence intensity in the marked area where the spark appeared. Fo is fluorescence intensity of the same cell area in the absence of Ca^{2+} spark. *Panel D:* Confocal line-scan image of a fluo-3-loaded control (WT) cell with the time course of Ca^{2+} sparks indicated below (upper panel). The fluorescence time course of the Ca^{2+} sparks was determined over the line indicated by the two arrows. Each line-scan image is a plot of fluorescence along a scanned line (ordinate) vs. time (abscissa). The line scan image duration was 5 s, and each line was 4 ms. Confocal line-scan image of a fluo-3-loaded SMAKO cell, with the time course of a Ca^{2+} spark indicated below (lower panel). Amplitudes of Ca^{2+} sparks are expressed as absolute values (delta$[Ca^{2+}]_i$) relative to the global resting cytosolic $[Ca^{2+}]_r$ at F0 using equation 1. *Panel E:* Comparison of spatial-temporal characteristics of Ca2+ sparks in WT and SMAKO cells.

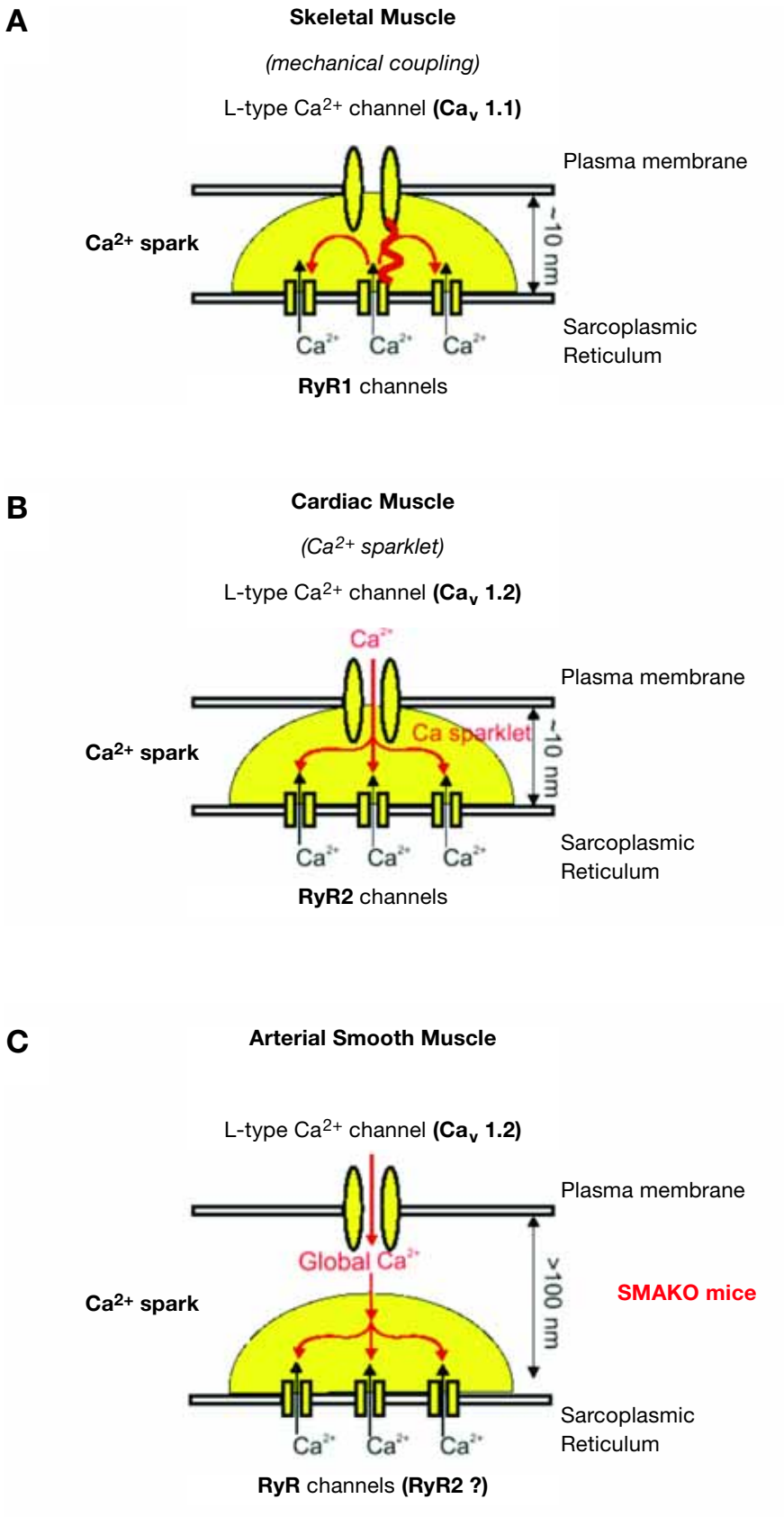

Figure 2

Role of $Ca_V1.x$ L-type channels in Ca^{2+} spark formation in muscle cells. *Panel A:* In skeletal muscle, the Cav1.1 L-type channel is physically coupled to one or multiple RyR1 channels in the SR (red wave-like line). Ca^{2+} efflux through RyR activates neighboring RyRs (red arrows). *Panel B:* In cardiac muscle, RyR2 channels are activated by local Ca^{2+} influx through an individual, co-localized (<10 nm) $Ca_V1.2$ L-type channel. This "local control of Ca^{2+}-induced Ca^{2+} release" underlies excitation-contraction coupling in cardiac muscle. *Panel C:* In arterial smooth muscle, our data indicate that $Ca_V1.2$ channels activate RyRs to release Ca^{2+} sparks mainly through control of the global cytosolic $[Ca^{2+}]_i$ indicating that local and tight coupling between the $Ca_V1.2$ channels and RyRs is not required to initiate Ca^{2+} sparks.

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Cardiovascular and Metabolic Regulation

Jens Jordan

The main interest of the group is basic (mechanism-oriented) patient-oriented research in the field of clinical autonomic disorders, arterial hypertension, obesity, and the metabolic syndrome. We run a clinical research center (CRC) that provides the infrastructure for these studies. One intention of our group is to combine patient-oriented research with basic science and genetics in the field of cardiovascular and metabolic diseases. The purpose of our research is to develop new treatment strategies for patients with obesity, the metabolic syndrome, arterial hypertension, orthostatic intolerance, and autonomic failure based on a better understanding of the pathophysiology of these conditions. We believe that studies on rare human diseases that are associated with low blood pressure may also yield important insight into the mechanisms of essential and obesity-associated hypertension. We are involved ongoing projects on the International Space Station (ISS). To elucidate the potential influence of candidate genes, we participate in twin studies together with HealthTwist. We have a close collaboration with other groups at the MDC to confirm hypotheses that are generated in humans in existing or in newly created animal models.

Autoimmune mediated autonomic failure

Over the last years, we established an internationally leading center on disorders of the autonomic nervous system. Autonomic failure is one of the conditions we are interested in. Autonomic failure patients suffer from severe orthostatic hypotension, anhydrosis, decreased saliva and tear production, impaired bladder emptying, and erectile dysfunction among other symptoms. In many patients, an underlying etiology cannot be found. A subgroup of patients with subacute autonomic neuropathy has serum autoantibodies against ganglionic acetylcholine receptors (AChR). However, a causal relationship between ganglionic AChR antibodies and autonomic failure has not been convincingly demonstrated in humans. We encountered a patient with long-standing severe autonomic neuropathy. Responses to autonomic function tests, baroreflex function, and plasma catecholamine concentrations were highly pathologic. He tested positive for ganglionic AChR antibodies. The antibodies blocked the intracellular signaling to acetylcholine in a dose dependent fashion (figure 1). Removal of antibodies by plasma exchange resulted in a dramatic clinical improvement. Furthermore, responses to autonomic function tests, baroreflex function, and catecholamine concentrations improved after treatment. Our study is the first to show that ganglionic AChR antibodies cause an endogenous ganglionic blockade. The important clinical implication is that patients with autonomic failure not otherwise explained should be tested for presence of AChR antibodies as a potentially treatable cause for autonomic failure. We plan additional experiments. We will try to transfer autoimmunity passively to a suitable animal model. Furthermore, we would like to develop more specific treatment strategies.

Adipose tissue as an endocrine and inflammatory organ

Adipose tissue secretes a large number of products that have been implicated in the pathogenesis of cardiovascular disease. We are particularly interested in adipose tissue-derived angiotensin II, leptin, and endocannabinoids. The renin-angiotensin-aldosterone system has been causally implicated in obesity-associated hypertension. We studied the influence of obesity and weight reduction on the circulating and adipose tissue renin-angiotensin-aldosterone system in menopausal women. We showed that obese women had higher circulating angiotensinogen, renin, aldosterone, and angiotensin-converting enzyme than lean women, and lower angiotensinogen gene expression in adipose tissue. A 5% weight loss in the obese group decreased circulating angiotensinogen, renin, aldosterone, and angiotensin-converting enzyme activity. Angiotensinogen expression in adipose tissue decreased markedly. The mechanisms may have a beneficial effect on blood pressure.

Leptin circulates in a receptor-bound and in a free form. We previously showed that bound and free leptin appears to have different biological functions. To further address the issue, we determined free leptin, bound leptin, and leptin receptor concentrations in monozygotic (MZ) and in dizygotic (DZ) twins. Our data are consistent with a strong genetic influence on both leptin receptor and bound leptin concentrations and a weaker genetic influence on free leptin concentrations.

Activation of the central endocannabinoid system increases food intake and promotes weight gain. We measured circulating endocannabinoid concentrations and studied the expression of CB1 and the main degrading enzyme, fatty acid amide hydrolase (FAAH), in adipose tissue of lean and obese women. Circulating levels of anandamide and 1/2-arachidonoylglycerol were increased in obese women. Adipose tissue mRNA levels of CB1 and FAAH were reduced in the obese group. FAAH expression in adipose tissue and circulating endocannabinoids were negatively correlated (figure 2). Expression of CB1 and FAAH was increased in mature human adipocytes compared to preadipocytes. Our findings support the presence of a peripheral endocannabinoid system that is upregulated in human obesity.

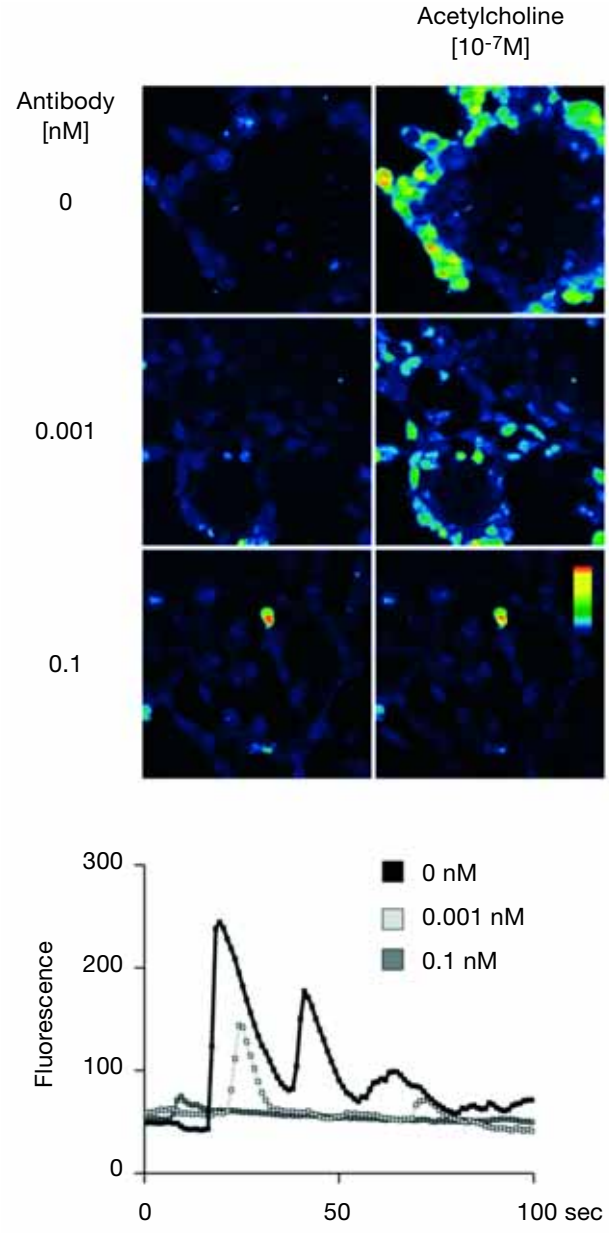

Figure 1
Effects of the patient's autoantibodies on intracellular calcium responses are displayed. Upper panel: IMR-32 cells before (left) and after (right) addition of 10-7 M acetylcholine in the presence or absence of serum with known antibody concentration. Lower panel: Typical time course of intracellular calcium concentration after addition of acetylcholine in a single cell. Addition of the antibodies attenuated the intracellular calcium response to acetylcholine in a concentration dependent manner.

Animal studies suggest that differentially expressed systemic activation of monocytes contributes to obesity-associated metabolic and cardiovascular complications. We tested the hypothesis that systemic monocyte activation is associated with changes in adipose tissue and skeletal muscle metabolism. Our data demonstrated that human monocyte activation is associated with tissue-specific changes in glucose and lipid metabolism. These findings may be explained in part by monocyte/macrophage infiltration of adipose tissue which appears to interfere with insulin responsiveness.

Novel pathways for lipid mobilization in humans

The goal of obesity treatment is to mobilize excess fat from adipose tissue. Traditional approaches to achieve lipid mobilization were focused on the adrenergic system. However, the utility is limited given the potential for cardiovascular side effects. In previous studies, atrial natriuretic peptide (ANP) in pharmacological concentrations was shown to stimulate lipid mobilization in humans. We tested ANP in physiologically relevant concentrations. Serum non-esterified fatty acids (NEFA) and glycerol concentrations increased dose dependently with ANP infusion. Using the microdialysis technique, we showed that ANP mobilized lipids in adipose tissue but not in skeletal muscle (figure 3). Thus, the natriuretic-peptide system may be a novel target for obesity treatment. Excess ANP release may contribute to cardiac cachexia.

Hypoxia training to "reprogram" oxidative metabolism and mitochondriogenesis in the metabolic sysndrome

Reductions in the number, aberrant location, and morphological changes of mitochondria have been described in insulin-resistant individuals. Microarray studies showed decreased expression of genes involved in oxidative phosphorylation in type 2 diabetic patients. These changes may be mediated through the PCG-1 pathway. The cellular energy sensor AMP kinase may also be involved. We hypothesize that stimulation of the system through hypoxia in combination with endurance exercise may activate mitochondrial biogenesis in adipose tissue and in skeletal muscle and reverse the metabolic abnormalities. We have recently established and opened a normobaric hypoxia program at our Clinical Research Center. This facility, coupled with our expertise in metabolic research, cardiovascular physiology, and in sports medicine, enables us to perform novel studies that cannot be performed elsewhere. Furthermore, our established program in obesity metabolic research at the molecular level gives us access to the necessary populations.

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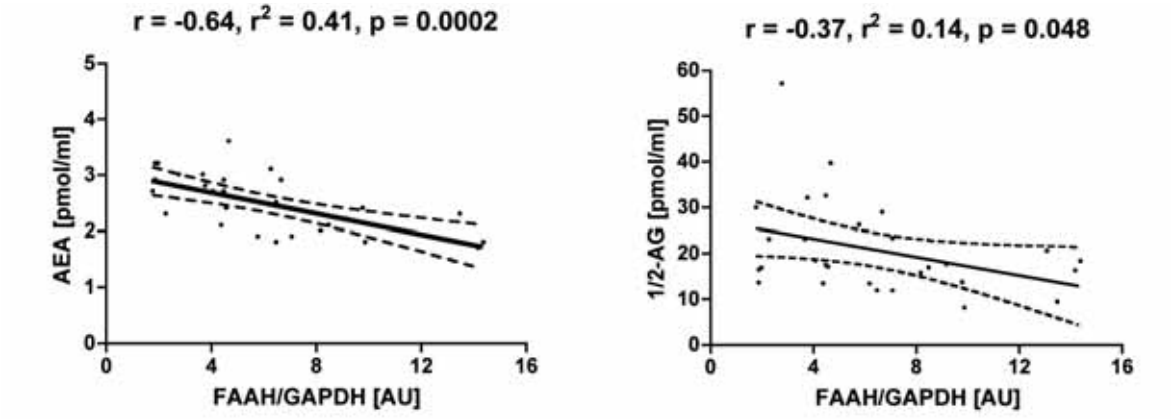

Figure 2 Circulating anandamide (AEA) and arachidonoylglycerols (1/2-AG) are negatively correlated with the expression of the FAAH gene in 40 human adipose tissue samples

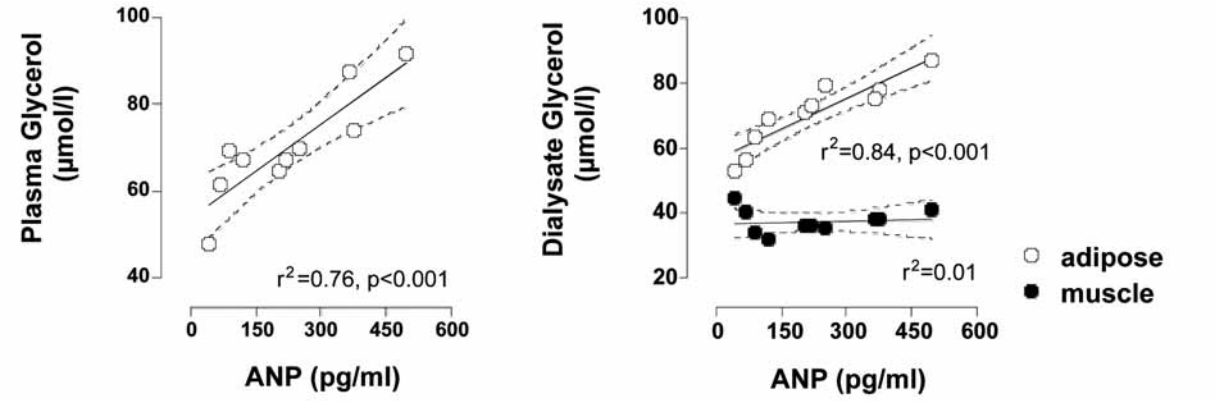

Figure 3 Correlation between serum ANP concentrations and venous NEFA concentrations and microdialysate glycerol concentrations in adipose tissue and skeletal muscle

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Functional Characterization of Newly Identified α Importins

Matthias Köhler
(Helmholtz Fellow)

Summary

The group is interested in nucleocytoplasmic protein transport. Various soluble transport factors, so called importins or karyopherins, mediate translocation of macromolecules into the cell nucleus. Importin α acts as an adapter by binding both the import substrate and importin β. The trimeric import complex docks to the NPC via importin β and translocates into the nucleus. After the cloning of several novel human β importins, our group continued to investigate the functional relevance of the various isoforms.

Alpha Importins

In collaboration with the group of Myriam Gorospe, Christina Quensel investigated the import pathway of HuR, a shuttling RNA-binding protein associated with reduced stability of its target mRNAs. She and her co-investigators showed that AMP-activated kinase (AMPK) plays a key role in the acetylation and phosphorylation of importin α1. Further results indicated that importin α1 is a key mediator of the AMPK-triggered HuR nuclear import.

Although previous *in vitro* studies suggested that the various α importins differ in their substrate specificity, their *in vivo* relevance was still unknown. Christina Quensel and Beate Friedrich therefore analyzed substrate specificity of human α importins in living cells via RNA interference. They were able to show that importin α3 is the only α importin responsible for nuclear import of RCC1 in living cells. Furthermore, down-regulation of importins α3, α5, and α7 strongly inhibited cellular proliferation whereas down-regulation of importins α1 and α4 had no or only minor effects.

Together with the group of Krister Melen, we further investigated the role of α importins in nuclear import of the transcription factor NF-κappaB. We showed that importin α3 directly binds to previously characterized nuclear localization signals of NF-κappaB p50 and p65 proteins. In this study, the binding site of importin α3 for NF-κappaB proteins was identified. The study showed that nuclear import of NF-κappaB is a highly regulated process mediated by a subset of importin α molecules.

Currently, Beate Friedrich, Franziska Hampich, and Tanja Schmidt are working in close collaboration with the groups of Michael Bader, Enno Hartmann, and Thomas Sommer on further identification of various *in vitro* and *in vivo* importin α a functions by the construction of several knock out mice.

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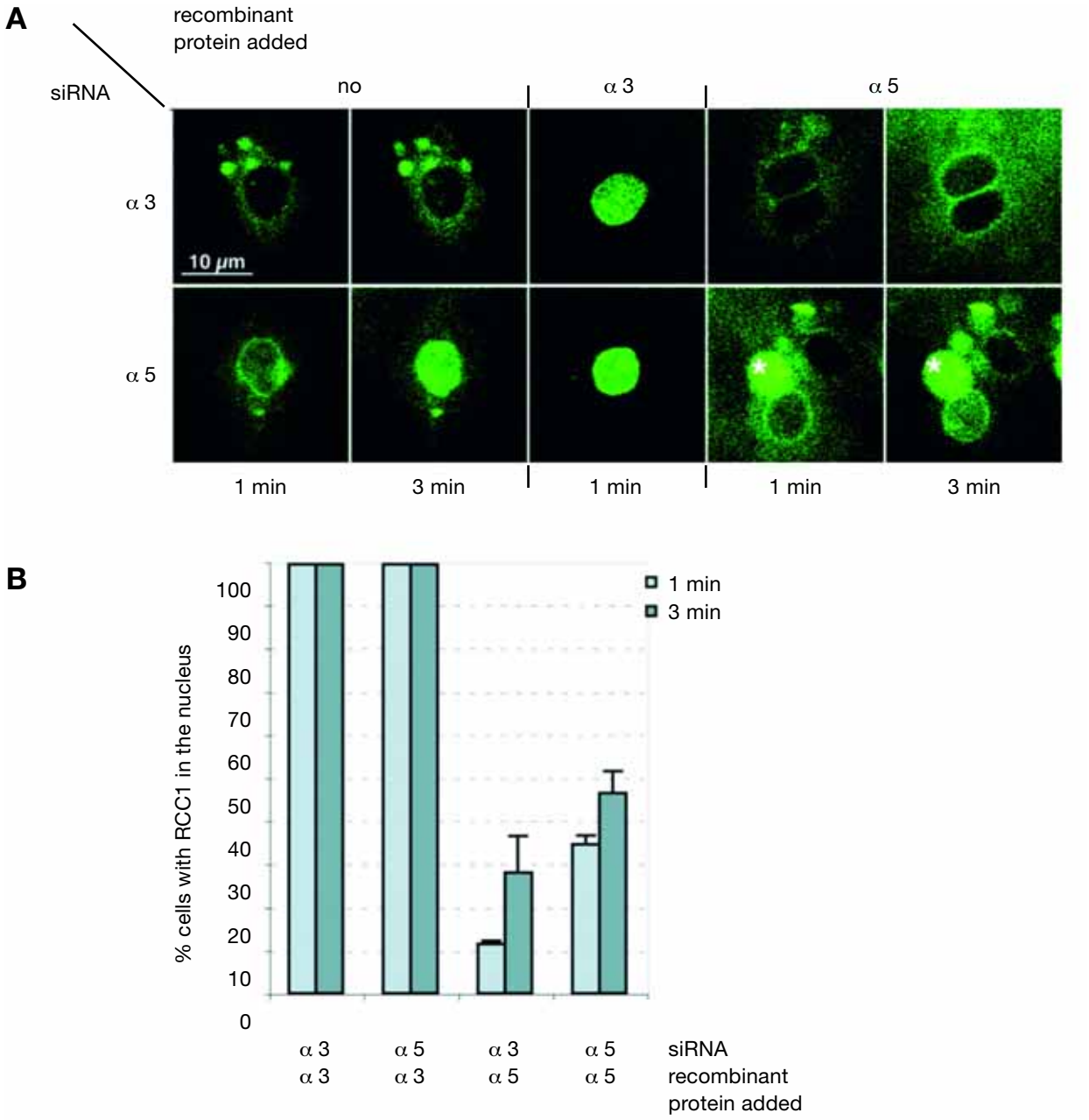


Coinjection of recombinant importin α3 but not of importin α5 restores nuclear RCC1 accumulation in HeLa cells with decreased expression of importin α3 or α5. **(A)** RCC1 was microinjected either alone or in combination with recombinant importin α3 or α5, and subcellular distribution was analyzed by confocal microscopy at the time points indicated. Representative images of two independent experiments analyzing at least 10 cells per experiment are shown. Stars indicate no nuclear accumulation, but artifact caused by injection. **(B)** Summary of the experiments described in the legend to panel A. Subcellular distribution of microinjected RCC1-fl was analyzed after 1 and 3 min by using confocal microscopy, and the percentage of cells with stronger nuclear than cytoplasmic staining was determined. Coinjection of importin α-3 caused fast RCC1 import, in less than 1 min, in 100% of importin α3 and α5 knockdown cells. Coinjection of importin α5 resulted in nuclear accumulation after 1 or 3 min in 12 or 28% and 35 or 47% of cells depleted of importin α3 or α5, respectively.

Mechanisms of Hypertension-induced Target Organ Damage

Dominik N. Müller
(Helmholtz Fellow)

Summary

The group has focused on hypertension/angiotensin (Ang) II-induced target organ damage. Dominik Müller was awarded a Helmholtz Fellowship in 2004. He collaborates with MDC scientists, as well as clinician-scientists from the Franz Volhard Clinic and elsewhere. The idea is to combine *in vitro* mechanistic studies, *in vivo* experimental animal studies, and patient-oriented research. Together with Ralf Dechend, Gerd Wallukat, and Duska Dragun, the group studied unique agonisitic antibodies capable of stimulating the Ang II AT1 receptor. They produced a novel model of preclampsia that develops these antibodies. Furthermore, they found that kidney-transplanted patients who develop C4D-negative humoral rejection also harbor these antibodies. Dominik Müller and colleagues produced findings similar to the human findings in transplanted rats infused with these antibodies. Other recent highlights include elucidation of PPAR-alpha and CYP2C23-dependent mechanisms in target organ damage, production of a cardiac cachexia animal model complete with gene expression, a study focused on the role of aldosterone in Ang II-induced target-organ damage, evaluation of a novel human renin inhibitor, and discovery that vascular smooth muscle cells express increased complement C3 levels when they exhibit a synthetic phenotype.

Cytochrome P450 (CYP) enyzmes and target organ damage

Dominik Müller has collaborated actively with Wolf-Hagen Schunck (MDC). In a DFG funded project, they studied the role of CYP enzymes and arachidonic acid (AA) metabolites in target-organ damage. They identified CYP2C23 as the major AA epoxygenase in the rat kidney. They also found that the PPAR-alpha agonist fenofibrate induced CYP2C23 activity. Chronic fenofibrate treatment cured double transgenic rats (dTGR) of Ang II-induced vasculopathy. As a result, fenofibrate induced renal microsomal epoxygenase activity which led to an increased 11,12-epoxyeicosatrienoic acid (EET) and hydroxy-epoxyeicosatrienoic acid (HEET) generation. The role of EETs and HEETs on cell proliferation and migration is the focus of present studies.

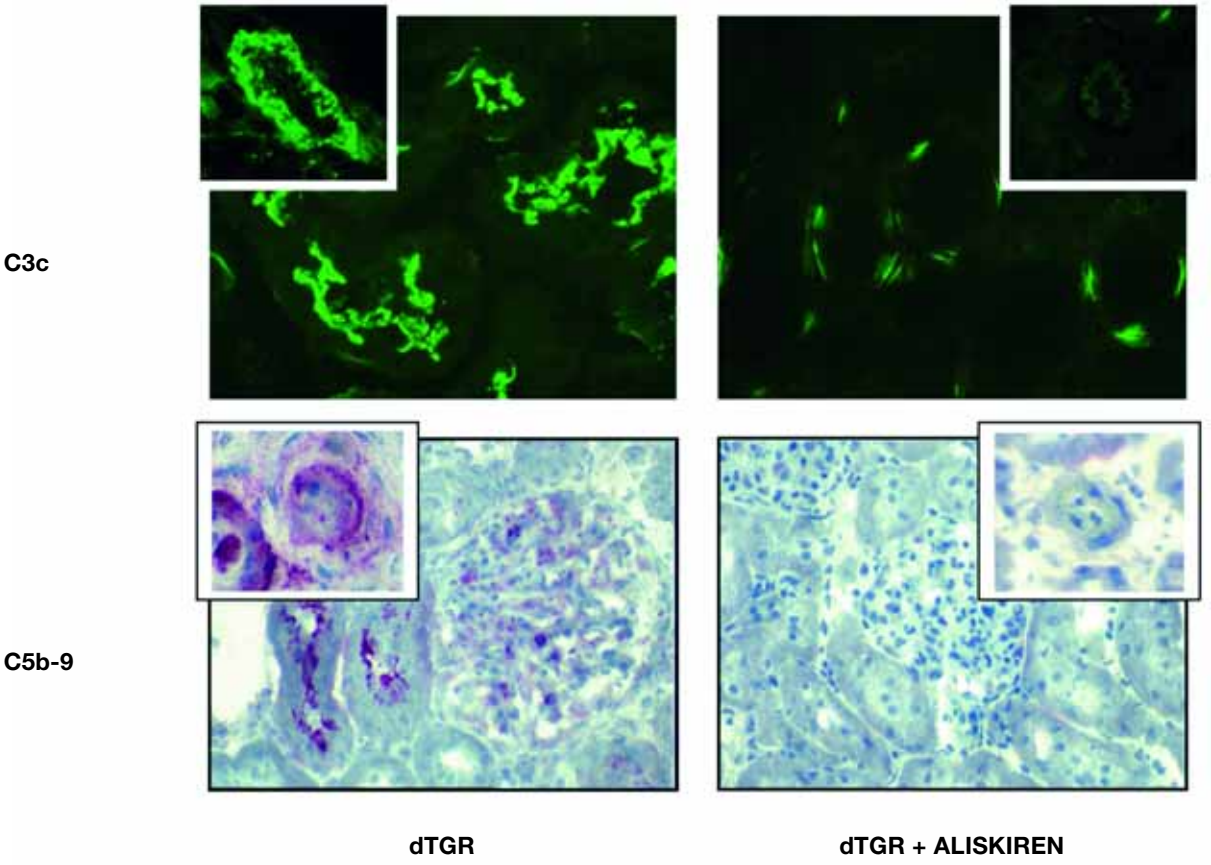
Agonistic AT1 receptor antibodies (AT1-AA)

Agonistic AT1 receptor antibodies (AT1-AA) were discovered in patients with malignant hypertension and preeclampsia by Gerd Wallukat (MDC) and collaborating clinicians from the Franz Volhard Clinic. Ralf Dechend and Dominik Müller observed that dTGR develop these same antibodies under certain circumstances. One example is when females harboring the human angiotensinogen gene are crossed with males carrying the human renin gene. While pregnant, the dams develop a preeclampsia-like syndrome featuring the antibodies. The rats exhibit proteinuria and renal histology very similar to human preeclampsia. Duska Dragun focused on kidney transplant patients who develop a humoral rejection that does not involve the complement protein C4d. She found that these patients also develop AT1-AA. An intense collaboration between Dominik Müller and the clincian-scientists culminated in a rat transplant model that provided strong evidence implicating AT1-AA in this form of rejection.

Cardiac cachexia, terminal heart failure, and gene expression
Half of untreated dTGR die at age week 7. The rats lose about a quarter of their body weight and exhibit severe cachexia with signs of terminal heart failure. Maren Wellner, Dominik Müller, and others performed a cardiac gene expression study and found that mitochondrial respiratory chain genes and lipid catabolism genes were reduced in expression while genes encoding transcription factors (CEBP-beta, c-fos, Fra-1), coagulation, remodeling/repair components (HSP70, HSP27, heme oxygenase), immune system (complement components, IL-6), and metabolic pathway were differentially expressed. Treating dTGR with losartan improved cardiac function and altered the gene expression patterns to those observed in non-transgenic healthy Sprague-Dawley control rats.

Role of aldosterone in Ang II-induced target-organ damage

Anette Fiebeler and Dominik Müller tested the hypothesis that reducing aldosterone by inhibiting CYP11B2 or by adrenalectomy (ADX) ameliorates target-organ damage in dTGR. In recent studies, they found that aldosterone plays a major role in the pathogenesis of end-organ damage. Inhibition of the mineralocorticoid receptor as well as blockade of the aldosterone synthase with the novel CYP11B2 inhibitor FAD286 ameliorates Ang II-induced renal and cardiac damage. Interestingly, removal of the adrenals results in an even more potent end-organ protection. The group also elucidated the source of cardiac aldosterone. They found that local cardiac aldosterone is produced in the adrenals. Another interesting finding is that aldosterone potentiates Ang II-induced signal transduction. The exact molecular mechanism is the focus of a current project. Altogether, the data indicate that crosstalk of Ang II and aldosterone play a key role in the pathogenesis of Ang II-induced organ damage.



The human renin inhibitor, aliskiren, reduces complement C3c and C5b-9 in angiotensin II-induced renal damage.

A human renin inhibitor

The renin-angiotensin system cascade would be best blocked at the renin step rather than at subsequent sites. However, the compounds had poor bioavailability which limited their use. Furthermore, the compounds could only be tested in primates, since the renins are species-specific. Aliskiren circumvents the bioavailability problem. The dTGR carry the human genes and, thus, provide an ideal animal model. The group studied aliskiren in the dTGR model. Aliskiren, in essence, eliminated hypertension and target organ damage in the dTGR model completely. Vascular smooth muscle cells isolated from dTGR exhibited a synthetic phenotype in that they grew faster and react differently in response to stimuli compared to wild type smooth muscle cells. One interesting finding involved the production of complement by the cells in response to TNF-α and C-reactive protein. The dTGR model showed strong evidence for complement activation and participation of the membrane attack complex in the production of vasculopathy (figure). These observations demonstrate the role of innate immune mechanisms that were hitherto fore not appreciated.

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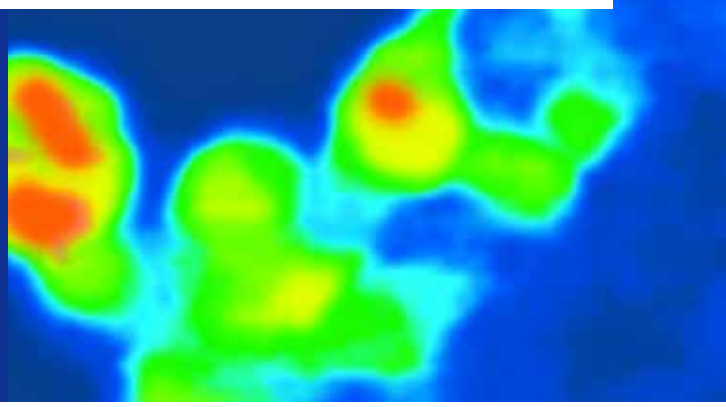


Cardiovascular Molecular Genetics

Ludwig Thierfelder

Arrhythmogenic Right Ventricular Cardiomyopathy and the Prevention of Sudden Cardiac Death

Sudden cardiac death (SCD) is a devastating complication in heart failure, a complex clinical syndrome with a myriad of etiologies. We study and search for genetic components of familial forms of heart failure in order to better understand structural and functional pathways of ventricular remodeling. Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a difficult-to-diagnose structural cardiac disorder associated with SCD and heart failure. ARVC is often familial in origin. In a large cohort of 120 ARVC patients, we identified mutations in plakophilin-2 *(PKP2)*, a component of the cardiac desmosome, in more than 25% of cases. The majority of *PKP2* mutations cause truncations of palkophilin-2 in these patients. In fact, western blot analysis of a cardiac biopsy from an ARVC patient with a *PKP2* deletion mutation revealed no abnormally sized plakophilin-2, suggesting haploinsufficiency as one operant mechanism. Although SCD in ARVC can occur in early adulthood, family members of ARVC index cases with *PKP2* mutations show a wide range of desease onset. Furthermore, disease penetrance in ARVC caused by *PKP2* mutations is less than 0.5.

SCD due to ventricular tachyarrhythmias can be successfully treated by the implantation of internal cardiac defibrillators (ICD). In a large founder population of ARVC patients from Canada, we have genetically mapped the defect to a 2 Mbp interval on chromosome 3p25 and haplotyped several hundred of these patients. As ARVC in this population is associated with a life expectancy of 39 years in males, we have started a program to identify mutation carriers by clinical and genetic analysis and provide those who carry the affected chromosome 3p25 ARVC haplotype with ICDs. We showed that this strategy can efficiently prevent SCD in these patients when compared to historical well matched but untreated controls.

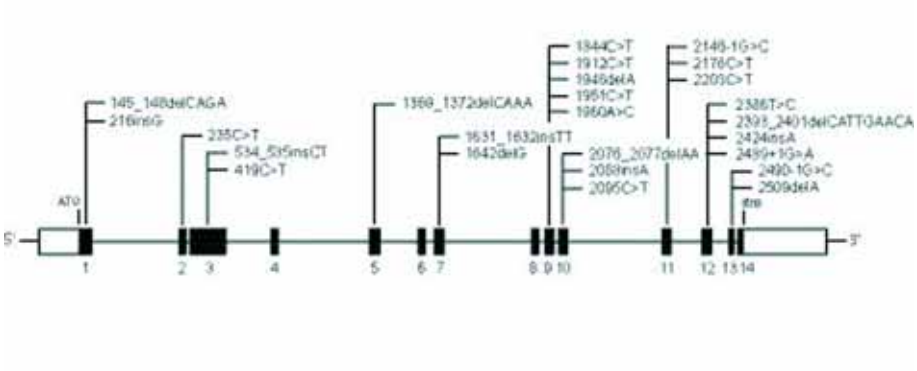
Molecular Genetic Studies in Other Forms of Familial Heart Failure

Isolated non-compaction of the left ventricle (INVC) is a rare disorder characterized by wide intertrabecular spaces due to an arrest of endomyocardial morphogenesis. It has been known for some time that infantile INVC is an X-chromosomal disease and is caused by mutations in G4.5, a gene with a yet unknown function. We studied a large population of adult INVC patients to assess whether genetic defects can also be accounted for in this population. In one large pedigree, INVC segregated as an autosomal dominant trait and a new locus on chromosome 11p15 was identified in a genomewide linkage analysis. Analyses of the putative disease gene are under way.

Dilated cardiomyopathy (DCM) in two large kindreds is associated with early onset of disease, sudden cardiac death, and diffuse myocardial fibrosis. DCM in these families is unlinked to the known DCM loci. In a genome wide screen, we identified a new tentative locus and are currently searching for the disease causing mutation.

Molecular Genetics of Pseudoxanthoma lasticum (PXE)

Pseudoxanthoma elasticum (PXE) 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 had previously mapped the PXE locus to a 500 kb interval on chromosome 16p13.1 and have since 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 types of mutations indicate loss-of-function as the genetic mechanism for the PXE phenotype. In 76 of the 81 families, the affected individuals were either homozygous for the same mutation or compound heterozygous for two PXE mutations. In the remaining five families with one uncovered mutation, affected individuals showed allelic compound heterozygosity for the cosegregating PXE haplotype. This demonstrates pseudo-dominance as the relevant inheritance mechanism, since disease transmission to the next generation always requires one mutant allelic variant from each parent. In contrast to other studies, our results show evidence only for recessive forms of PXE.



Partial schematic of the plakophilin-2 gene *(PKP2)* with ARVC causing mutations. In a mutation screen of 120 ARVC patients, 25 different *PKP2* mutations were identified in 32 index cases. The majority of the *PKP2* mutations introduce premature stop signals causing trunctions of plakophilin 2. Three *PKP2* mutations have been identified in several independent cases.

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Genetic Disorders of the Cardiovascular System

Brenda Gerull
(Helmholtz Fellow)

Genetic causes of heart failure and sudden cardiac death

Congestive heart failure is a complex syndrome resulting from various disease states with inadequate cardiac output. Familial forms of congestive heart failure can be studied by genetic analyses. Cardiomyopathies (CMPs) are heart muscle disorders with a strong genetic component. Dilated cardiomyopathy (DCM) is an etiologically heterogeneous cardiac disease characterized by left ventricular dilatation and systolic dysfunction. Approximately 25 to 30% of DCM patients show a family history of autosomal dominant inheritance. We have demonstrated that mutations in the giant muscle filament titin (TTN) can cause DCM. The prevalence of TTN mutations in familial DCM is unknown.

TTN encodes for the largest known protein, titin. Titin serves as a scaffold in the sarcomere, plays a role in myofilament turnover and, probably, in myocyte signal transduction. More than 360 exons code for multiple alternatively spliced isoforms of approximately 1-3MDa in size which are expressed in cardiac and skeletal muscle. A role for titin in non-muscle tissue (where it may be expressed at low levels) is less clear. Because of the extensive genomic sequence, studies to screen the entire coding sequence for mutations in larger populations are not feasible. However, due to its enormous size and multiple functions, titin is a prominent target for mutations and may account for a significant proportion of the genetic etiology of familial DCM.

In a large DCM kindred, a segregating 2bp insertion mutation (c.43628insAT) in titin exon 326 causes a frame shift, thereby truncating A-band titin. The truncated ?2MDa protein is expressed in skeletal muscle but Western blot studies with epitope-specific anti-titin antibodies suggest its proteolytic processing into a 1.14MDa subfragment by site-specific cleavage within the PEVK region. Interestingly, in a cardiac biopsy sample taken from an affected patient, the truncation mutation seems not to be expressed (or actively degraded) at the cDNA or protein level. A mouse model expressing the truncation mutation should provide further insight.

In two other large DCM families, linked to chromosome 2q31, we have found mutations in TTN: a missense mutation, W930R, is predicted to disrupt a highly conserved hydrophobic core sequence of an immunoglobulin fold located in the Z-disc/I-band transition zone, and a frameshift mutation, K20963fsX20972, which results in a premature stop codon and truncates the A-band titin. Further linkage studies of families and more efficient screening strategies in larger populations will be required to survey the frequency of titin mutations in inherited forms of DCM. In addition, further investigations of molecular pathways will provide new insights into pathogenetic mechanisms of DCM caused by TTN mutations.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a genetically heterogeneous heart muscle disorder clinically characterized by ventricular arrhythmias and heart failure. We have identified a common disease gene for ARVC. Heterozygous mutations in *PKP2*, which encodes plakophilin-2, an arm-repeat protein of the desmosome, cause more than 25% of ARVC cases. Genes encoding other junctional components than PKP2 will be tested for the presence of mutations in a large cohort of ARVC patients.

Genetics of antiphospholipid antibody syndrome and thrombozytopenia linked to chromosome 10p12

The antiphospholipid antibody syndrome (APS) is a complex, usually aquired hypercoagulation disorder, clinically characterized by thromboembolism, stillbirth, and thrombozytopenia in the presence of antiphospholipid antibodies. It is largely unknown whether genetic factors play a role in this complex syndrome. We have identified a large kindred (A) with multiple members clinically affected by one or more features of APS. Segregation, linkage, and molecular analyses have identified at least two genetic defects in Kindred A, one being a factor V Leiden mutation in a nuclear pedigree of kindred A and a yet unknown defect on chromosome 10p12 causing thrombozytopenia. Other APS features in kindred A (e.g., presence of antiphopholipd antibodies, stillbirth) have not yet been mapped. We are currently screening for the chromosome 10p12 gene carrying the mutation responsible for thrombozytopenia in kindred A.

Selected Publications

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Myocardial Regeneration and Heart Failure

Rainer Dietz

The cardiovascular research group of Rainer Dietz consists of three distinct subgroups focussing on myocardial regeneration and cardioprotection, on heart failure and congenital heart disease, and on molecular mechanisms of preeclampsia. For a better presentation of the different research activities, the groups will be described separately as follows:

1. Myocardial Regeneration and Cardioprotection

When fully differentiated, the mammalian heart is composed of cardiomyocytes which have withdrawn from the cell cycle. Thus, the heart is not able to compensate for cell loss rendering it biologically inert in regard to regeneration.

Inducing mitosis and cytokinesis in postmitotic cardiomyocytes

Cardiomyocytes lose their ability to divide during early neonatal period attaining a terminally differentiated state. We hypothesized that ectopic expression of cell cycle factors promotes cardiomyocyte division. Isolated postnatal and adult rat ventricular cardiomyocytes were adenoviral transfected with E2F2, Cyclin D1 and cdk4 either alone or in combination. 72 hours after transfection, cells were prepared for indirect immunocytochemistry using antibodies against phosphorylated histone H3 (PiH3) for monitoring mitotic cells. Additionally antibodies to alpha tubulin in conjunction with PiH3 were employed for detecting cardiomyocytes undergoing cytokinesis. Each of the cell cycle factors employed is capable of inducing mitosis in neonatal cardiomyocytes on its own. However adult cardiomyocytes require their combined activity.

In conclusion, our study provides sustained evidence that terminally differentiated cardiomyocytes can divide when challenged with cell cycle activating factors.

2. ARC is required for cardioprotection in response to biomechanical and ischemic stress

ARC is a master regulator of cardiac death signaling, as it is the only known factor that specifically inhibits both apoptotic death pathways. By generating ARC deficient mice, we attempted to elucidate the physiological role of ARC. ARC null mice developed normally to adulthood and had no abnormality in cardiac morphology and function under resting conditions. 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, we 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.

3. Clinical and Molecular Research in Heart Failure and Congenital Heart Disease

CardioGenetic Laboratory at the Clinical Research Center

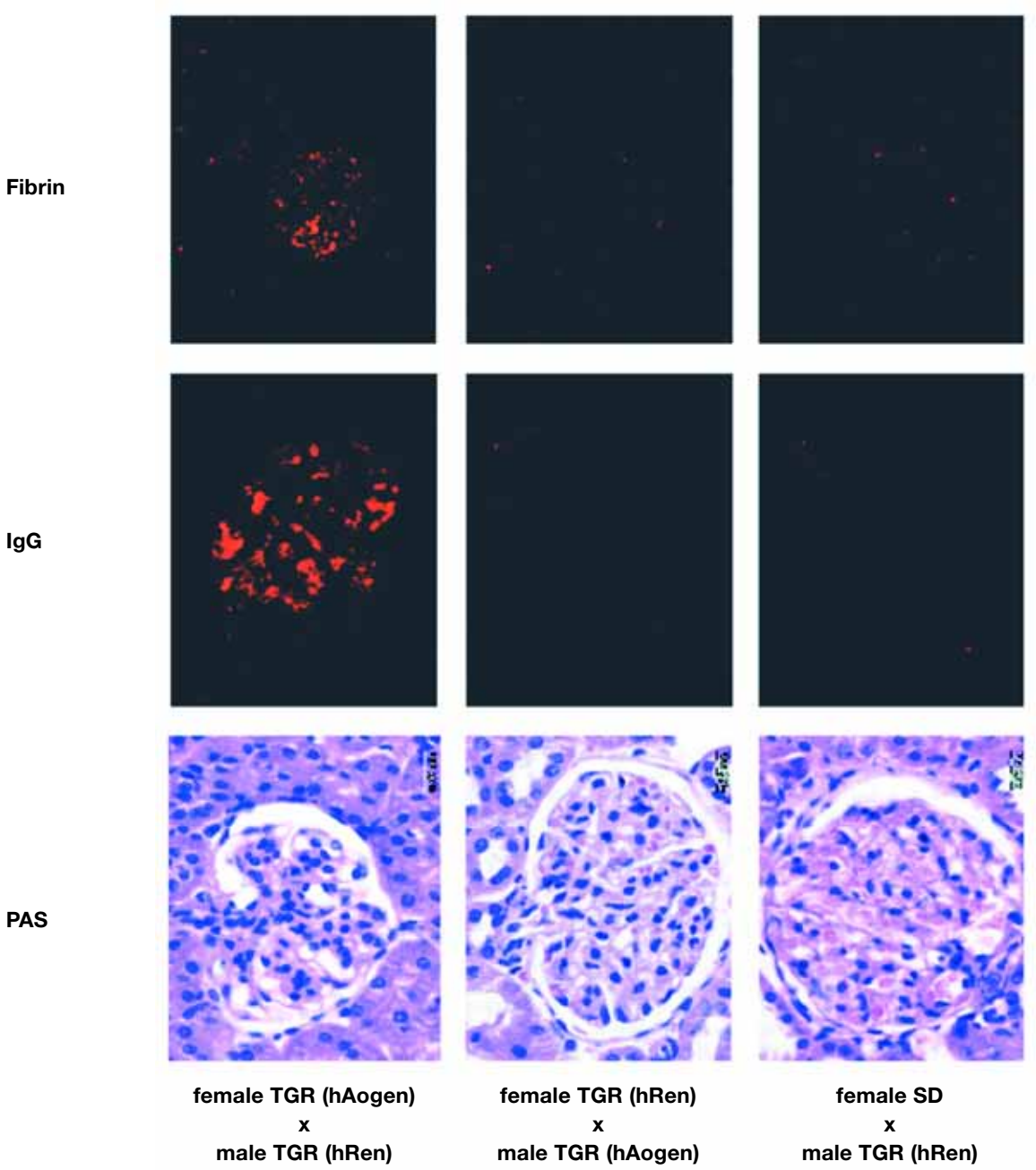
The group is focussed on “disease oriented research” in the field of heart muscle diseases. We are mainly interested in the pathogenesis and molecular mechanisms of cardiomyopathies and heart failure, especially inherited forms of hypertrophic and dilated cardiomyopathy are examined in different projects. A further area of interest is genetic aspects of congenital heart diseases where we cooperate with the pediatric cardiologists from Charité and the German Heart Center Berlin. Genetic analysis of patients is done using candidate gene approach and linkage analysis (in close cooperation with the MDC Gene Mapping Center). A number of mutations in different genes were detected. Recently, we were able to identify a new disease gene for cardiomyopathy, the muscle LIM protein (MLP) gene.

4. Molecular mechanisms of preeclampsia

Preeclamspia afflicts 5% of women in Europe. The condition is the commonest cause of morbidity and mortality for both mother and child in the perinatal period.

Preeclampsia at the Franz Volhard Clinic

Ralf Dechend, Volker Homuth, Dominik N. Müller, Gerd Wallukat, and Friedrich C. Luft showed that AT1-AA and Ang II both stimulate the AT1 receptor and initiate a signaling cascade resulting in tissue factor expression. Their results showed an action of AT1-AA on human cells that could contribute to the pathogenesis of preeclampsia. The group next showed that the NADPH oxidase, the major producer of reactive oxygen species (ROS) in blood vessels, is potentially an important source of ROS that may upregulate NF-kappaB in preeclampsia. They suggested that AT1-AA through activation of NADPH oxidase could contribute to ROS production and inflammation. These provocative findings have since been confirmed by the group of Rodney Kellems from the University of Texas, Houston, TX, USA.



Immunofluorescence for fibrin, IgG, and periodic acid Schiff (PAS). The female hAogen x male hRen cross showed glomerular staining, whereas the other crosses did not. Similarly, a PAS stain showed evidence of endothelial cell swelling (endotheliosis) in the affected cross.

Ralf Dechend works closely with Volker Homuth, and Friedrich C. Luft at the Franz Volhard Clinic and with Dominik N. Müller and Gerd Wallukat at the MDC. This project is an example of interdisciplinary research that the Franz Volhard Clinic (Departments of Cardiology and Nephrology and MDC) are pledged to uphold.

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Summary

The Cardiovascular Magnetic Resonance (CMR) group at the Franz Volhard Clinic has focused its research on the *in vivo* assessment of functional and structural myocardial abnormalities related to inflammatory diseases and coronary heart disease. Using two 1.5 clinical MRI-scanners with dedicated cardiac software, we developed new approaches for the differentiation of tissue changes in myocardial diseases. The application of this equipment as a research tool and the translation into a clinical setting are the main interests of the group.

Inflammation

The diagnosis of myocarditis and the assessment of myocardial tissue changes during follow-up are challenging tasks in cardiovascular research and clinical cardiology. Clinical presentation of patients with myocarditis 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 1990s, we developed an approach for the non-invasive detection of acute myocarditis by CMR. In 2005, we published a new multi-sequential approach with a significantly increased diagnostic accuracy. Myocardial inflammation also has a high prognostic impact in different systemic diseases. However, early assessment is difficult. We used CMR technology in patients with sarcoidosis and were able to detect myocardial involvement in those patients with preserved left-ventricular function. Research in Churg Strauss syndrome and amyloidosis is ongoing. The current multi-sequential approach allows the quantitative assessment of edema, as well as the detection of reversible and irreversible contrast-enhancement (corresponding to hyperemia, capillary leakage, and fibrosis, respectively). Further technical developments will improve the diagnostic accuracy of diffuse homogeneous abnormalities of the myocardium. A new pulse sequence which enables the direct quantification of magnetic relaxation properties of the myocardium was generated and is now implemented. The diagnostic value of this new method will be studied in various clinical settings, with a focus on patients with inflammatory diseases.

As known, inflammation also can impair endothelial function. We showed with standard tests for endothelial dysfunction that blood-oxygen-level-dependent (BOLD) MRI can detect dissociation of tissue hemoglobin oxygenation from blood flow. BOLD MRI may be a useful adjunct in assessing endothelial dysfunction.

Coronary Artery Disease and Arteriosclerosis

The detection of coronary artery stenosis is a growing field in CMR. We compared the performance of a contrast-enhanced to a non-contrast breath-hold 3D-SSFP-pulse sequence and showed that extensive parts of the coronary arteries are depictable without application of contrast media. The need to differentiate acute from chronic and irreversible myocardial injury, as well as to evaluate the impact of myocardial injury, is a common challenge in clinical decision-making and often represents a limit for currently available imaging modalities. We have shown that combined sequences offer the capability to differentiate acute from chronic myocardial infarction and the impact of reperfusion injury after acute myocardial infarction. Elective percutaneous interventions are associated with intermittent impairment of myocardial perfusion and its impact on prognosis and therapeutic consequences are under investigation. It is known that the degree of a vessel stenosis is not a strong marker for vulnerability. A new field in CMR is the work on the atherosclerotic plaque differentiation. We started to assess plaque morphology in the carotid artery. In a clinical setting, we identified MR signals suggestive of vulnerable plaques, a finding which was independent of the degree of stenosis.

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Myocardial Signal Transduction in Heart Failure

Martin W. Bergmann
(Helmholtz Fellow)

Heart failure is the leading cause of hospitalization in western civilized countries. In recent years, the balance between the three cellular phenomenons of programmed cell death (apoptosis), cardiomyocyte hypertrophy, and possibly regeneration has been determined as the cellular basis of heart failure development. The current pharmacological treatments rely on the unspecific blockade of cell-surface receptors previously determined to negatively influence the heart's adaptation to chronic stress after myocardial infarct or pressure overload in arterial hypertension. Recent genetic approaches have centered around specific intra-cellular signaling cascades mediating particularly cardiomyocyte hypertrophy, which precedes the development of open heart failure. Most prominently, the calcium-controlled transcription factors NF-AT and MEF2 have been identified as mediators of cardiac mal-adaptation.

The Bergmann group located at the Max Delbrück Center of Molecular Medicine and closely linked to the Franz Volhard Clinic, Charité Campus Berlin-Buch, has focused on the role of other specific transcription factors involved in cardiac hypertrophy. Techniques used include both isolated neonatal and adult primary cardiomyocytes as well as transgenic mice generated by Cre/lox techniques. Similar to NF-AT, the transcription factor NF-κB was first identified for its prominent role in regulating inflammatory cytokines. However, NF-κB was recently found to be important for B-cell proliferation in Hodgkins's disease and shown to be necessary for G-protein coupled receptor – induced cardiomyocyte hypertrophy in vitro as well as survival of cardiomyocytes. Therefore the group tested the hypothesis, that NF-κB is also involved in cardiomyocyte growth. Similarly, the transcription factor cAMP response element binding protein (CREB) was studied in cardiomyocyte hypertrophy induced by transient hypoxia. In order to translate these findings to patient treatment, the effect of currently used drugs on the activity of these transcription factors was investigated. The studies have established a role of these two factors in maladaptive cardiac remodelling, which is now further investigated.

NF-κB is a suitable target to improve left ventricular remodeling in vivo

Mice with heart-specific NF-κB inhibition were generated by mating α-myosin heavy chain Cre-recombinase mice (Michael D. Schneider, Houston, Texas) to loxP-IκBΔN mice in collaboration with R. Schmidt-Ullrich, group Scheidereit (MDC). The mice have been characterized at baseline as well as after 14 days of AngII infusion by osmotic minipumps. Histologic, echocardiography, and gene expression analysis revealed diminished hypertrophy in mice with heart-specific NF-κB inhibition compared to control animals. Gene chip analysis comparing adult cardiomyocytes with adenoviral overexpression of NF-κB inhibitor IκBΔN to control virus transfected cells revealed a set of NF-κB targets, which seem to be heart specific as a control of these genes by NF-κB has not been described before. One of these target genes, the IL-6 cytokine receptor gp130, has been validated in the above mentioned mouse model and could explain the beneficial effects of NF-κB inhibition on heart remodelling. After finishing this part of the study the group now aims to study the effect of NF-κB inhibition in heart failure development after chronic pressure overload by transverse aortic banding. The hypothesis that NF-κB inhibition might serve as a new strategy in preventing heart failure development and/or worsening will be tested.

CREB is essential for hypoxia/reoxygenation induced cardiomyocyte hypertrophy

Another set of experiments has focused on cardiomyocyte hypertrophy induced by hypoxia followed by reoxygenation similar to the ventricular remodeling observed *in vivo* after myocardial infarct. While hypertrophy was not altered by inhibiting NF-κB activation, a role for CREB downstream of the PI3-kinase/AKT/GSK3β signaling pathway was identified in these studies. Interestingly, GSK3, did not alter CREB serine133 phosphorylation, the common endpoint of CREB stimulation regulating transactivation of CREB-responsive genes. Instead, GSK3, regulated CREB DNA binding, possibly by a second phosphorylation at CREB serine 129.

Statins protect cardiomyocytes from apoptosis by inactivating GSK3,

Our data imply differential sets of transcription factors involved in cardiac remodeling preceding heart failure. These studies prompted us to investigate the effect of currently used drugs on cardiomyocyte nuclear signaling. Statins are used for their effect on cholesterol levels in blood. However, recent evidence suggests a direct effect on cardiac remodeling independent of vascular protection. Experiments with isolated rat cardiomyocytes revealed activation of the well-known PI3-kinase/AKT/GSK3, pathway resulting in reduced apoptosis. Downstream of GSK3,, the transcription factor β-catenin was stabilized as an effect of statin treatment

Selected Publications

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Molecular Muscle Physiology

Ingo L. Morano

Contractility of cardiac and smooth muscle is regulated by calcium ions (Ca^{2+}) which enter the cells through voltage-gated L-type Ca^{2+} channels and, subsequently, induce the release of high amounts of Ca^{2+} from the sarcoplasmic reticulum into the myoplasm through calcium release channels. Ca^{2+} activates both intracellular signalling pathways and contraction of the myofibrils. In cardiomyocytes, Ca^{2+} activates the myofibrils by binding to troponin C, which turns the thin filament from an "off" into an "on" state, allowing the molecular motor myosin to interact with the thin filament to produce force and shortening. In smooth muscle cells, Ca^{2+} forms a complex with calmodulin which activates the myosin light chain kinase, an enzyme which phosphorylates a 20kDa regulatory light chain of myosin, thus allowing the smooth muscle myosins to generate contraction upon interaction with the thin filaments. Because of their key-roles in muscle, we are studying the expression regulation, post-translational modifications, and functional roles of the subunits of L-type Ca^{2+} channel, proteins of the Ca^{2+} signalling pathways, and Type II myosin in cardiac and smooth muscle. Any change in these key proteins, by mutation, differential gene expression, alternative splicing of the transcripts, or post-translational modification, modulates cardiac and smooth muscle function. Understanding muscle contraction regulation at the molecular and functional levels provides an opportunity to develop new therapies for the treatment of cardiac and smooth muscle dysfunction.

Atrial essential myosin light chain invigorates cardiac contractility

Type II myosin isoenzymes are hexamers of about 500 kDa composed of two heavy chains (MyHC) and 4 light chains (MLC). Atrium- and ventricle-specific essential (ALC-1 and VLC-1, respectively) and regulatory (ALC-2 and VLC-2, respectively) MLC exist in the human heart. Cardiomyocytes of hypertrophied human ventricles re-expressed ALC-1, while MyHC isoenzymes did not change. Ventricular *in vitro* preparations with myosin associated with ALC-1 revealed a higher force generation, shortening velocity, rate of force development as well as Ca^{2+} sensitivity than normal ventricular preparations without ALC-1. Ca^{2+}-calmodulin dependent second messenger pathways, in particular calcineurin-NFAT and CaMK IV were involved in the activation of the human ALC-1 promoter. Transgenic overexpression of the human ALC-1 in the rat heart significantly improved the contractility of the whole isolated perfused, electrically driven heart.

Elucidation of the latch-state: Non-muscle myosins are the latch-bridges

Sustained activation of smooth muscle elicits an initial phasic contraction and a subsequent tonic contraction. Tonic contraction of smooth muscle is unique since it is generated at almost basal free Ca^{2+}, reduced oxygen consumption, ATPase

activity, and shortening velocity ("latch state"). The latch mechanism remained obscure for decades. Smooth muscle cells express three MyHC genes, namely one smooth-muscle-specific (SM-MyHC) as well as two non-muscle-MyHC (NM-MyHCA and NM-MyHCB). We eliminated expression of the SM-MyHC by gene targeting technology. Smooth muscle from knock-out neonatal mice did not exhibit initial phasic contraction while tonic contraction remained normal. Intracellular Ca^{2+} transients of smooth muscle cells from wild-type and knock-out animals were similar. Thus, initial phasic contraction is generated by SM-MyHC recruitment while the sustained tonic contraction state can be produced by NM-MyHC activation, which represents the latch cross-bridges in smooth muscle.

Elucidation of the missing link: Ahnak confers the sympathetic stimulation of cardiac L-type calcium channel activity

Sympathetic tone is a major determinant of the L-type calcium channel activity, thus regulating influx of calcium ions (Ca) from exterior into the cytosol of cardiomyocytes (ICaL). Sympathetic stimulation of ICaL is known to be mediated by a cascade of reactions involving beta-adrenergic receptors, G-protein coupled adenylyl cyclase, and protein kinase A (PKA). The molecular basis of this regulation, in particular the site(s) targeted by PKA, as well as the mechanism by which phosphorylation increased IcaL, remained obscure. In fact, the postulated link between Cav1.2 phosphorylation and enhanced ICaL could not be demonstrated in the Xenopus oocyte expression system. Thus, PKA-dependent regulation of IcaL may require additional unidentified components. Searching for those "missing links" led us to the identification of the 700kDa phosphoprotein ahnak in mammalian cardiomyocytes which could initially be characterized by co-precipitation with the β2 subunit and *in vivo* phosphorylation in response to sympathetic stimulation of the heart.

Ahnak is encoded by an intronless gene on human chromosome 11q12-13. In the myocardium, we localized ahnak to cardiomyocytes, endothelial cells and smooth muscle cells of blood vessels. At the subcellular level, ahnak locates to the cytoplasmic aspect of the sarcolemma in cardiomyocytes. It

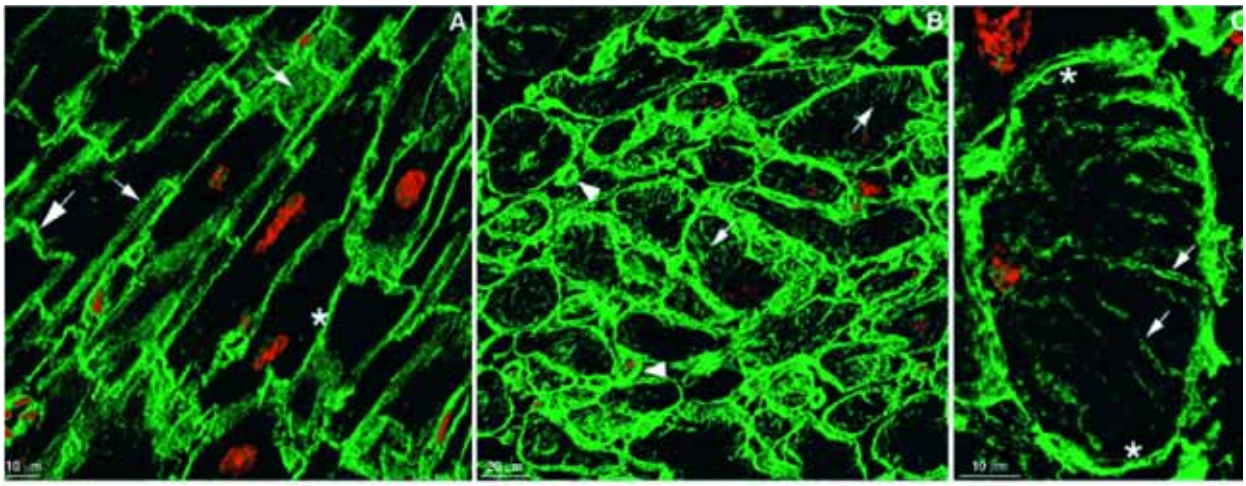

Figure 1
Confocal images depicting the localization of ahnak-C2 in the human heart. Longitudinal (A) and cross (B) sections of human myocardium were stained for ahnak-C2 (green) and nuclei (red). Ahnak-C2 labels the T-tubular system (small arrows), the surface sarcolemma (star), and the intercalated discs (big arrow) C) High magnification of a transversal section showing one myocyte. The T-tubular system (arrows) is oriented radially inside the myocyte (from: Hohaus et al. 2002, FASEB 16: 1205-1216).

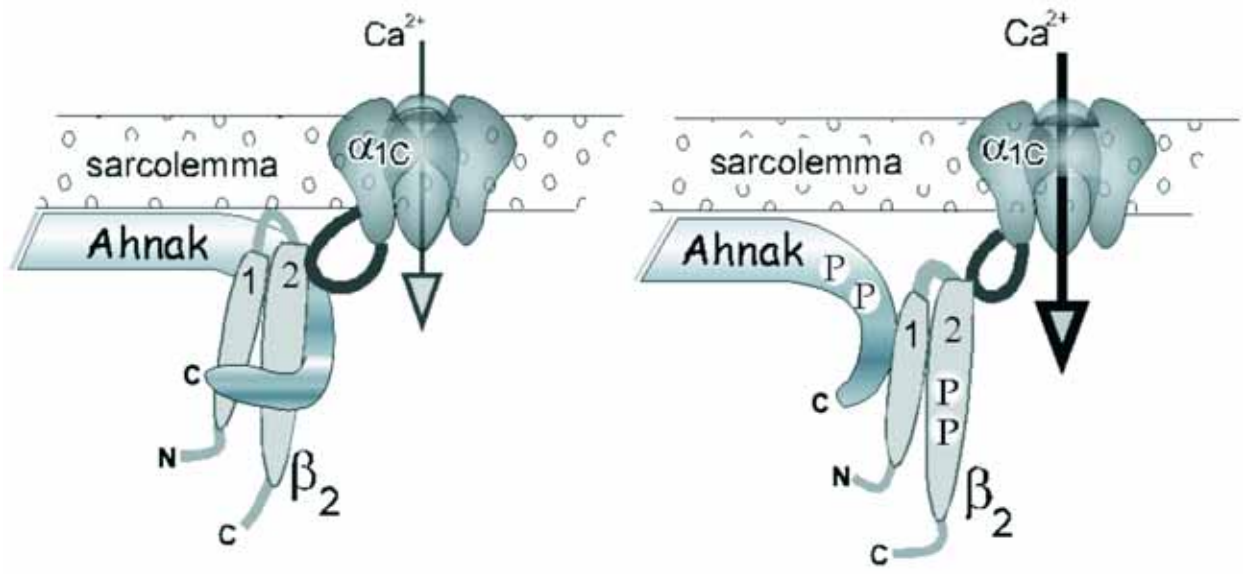

Figure 2
Proposed model for sympathetic control of ICaL by ahnak/Ca^{2+} channel binding. Under basal conditions, ICaL carried by the ß1C-subunit is reprimed by strong ahnak-C1/ß2-subunit binding (left panel). Upon sympathetic stimulation, PKA sites in ahnak and/or in ß2 become phosphorylated. This attenuates ahnak-C1/ß2-subunit binding resulting in increased ICaL since ß2-subunit is more available for ß1C (right panel). Hence, we propose ahnak-C1/ß2-subunit binding serves as physiological inhibitor of ?1C conductance. Relief from this inhibition is proposed as pathway used by the sympathetic signal cascade. Likewise the missense mutation Ile5236Thr attenuated ahnak/ß2 interaction thus increasing ICaL.

interacts with the channel β2-subunit via multipoint attachment mediated by ahnak's carboxy-terminal domains, ahnak-C1 and ahnak-C2. The most C-terminal ahnak-C2 domain has actin-binding and actin-bundling capacity. As such it provides a link to the subsarcolemma cytoskeleton and stabilizes muscle contractility. Recent patch-clamp experiments on rat ventricular cardiomyocytes showed that targeting the high affinity ahnak-C2/β2-subunit interaction by a peptide competition approach leads to an increase in the Ca^{2+} current amplitude and a slowing of channel inactivation. These results suggested that endogenous ahnak exerts a sustained inhibitory effect on ICaL by strong β2-subunit binding via the ahnak-C2 domain. Furthermore, the interaction between ahnak-C1 and β2-subunit plays a critical role for the sympathetic regulation of L-type Ca^{2+} channel activity: PKA phosphorylation significantly reduced interaction between ahnak-C1 and the β2-subunit, thus relieving its inhibitory effect on IcaL.

We screened a patient cohort with hypertrophic cardiomyopathy in order to identify naturally occurring, genetic ahnak variants. The identification of the coding genetic variant Ile5236Thr-ahnak prompted us to study functional consequences of this mutation on β2-subunit binding and Ca^{2+} channel function. We found that Ile5236Thr ahnak interfered with the classic beta-adrenergic regulation of IcaL.

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Cell Polarity and Epithelial Formation

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Summary

Epithelial cells polarize along their apico-basal axis and separate apical from basolateral membrane compartments during development. Mature epithelial cells are highly polarized with separate apical and baso-lateral membrane compartments, each with a unique composition of lipids and proteins. Within mature epithelial tissues, cell polarity regulates cellular morphology, intracellular signaling, asymmetric cell division, cell migration, and cellular and tissue physiology as well as complex organ morphogenesis. We are interested in the molecular mechanisms that regulate the polarization of epithelial cells and are using zebrafish and fruitfly *Drosophila* as our experimental systems. We would like to understand: How do the different protein complexes that establish cell polarity interact with each other? What are the signals by which cell polarity is mediated within cells? How is cell polarity regulated within epithelial sheets during morphogenesis of tissues and organs? How is cell polarity linked to the morphogenesis of the early zebrafish heart? Several zebrafish mutants with defects of epithelial cell layers will help us to address this issue. Our long-term interest is to understand how the cellular mechanisms controlling cell polarity shape our own bodies.

Recent Projects

Cell polarity and zebrafish cardiac morphogenesis

Vertebrate heart morphogenesis involves the generation of a simple tube that is shaped into a complex functional organ. The types of cellular and tissue rearrangements driving this process are largely unknown. In zebrafish, heart morphogenesis involves fusion of two bilateral populations of cardiac progenitor cells at the midline of the embryo followed by the formation of the three-dimensional heart cone, a transitional structure that consists of a dorsal ventricular and a ventral atrial part. Subsequently, the heart cone moves from the dorsal-ventral into the anterior-posterior plane, a process referred to as tilting. As a consequence, the ventricular portion of the heart cone comes to lie more posteriorly. Finally, the heart cone elongates along the anterior-posterior axis into the heart tube that is converted into a looped, two-chambered organ.

The zebrafish mutation *heart and soul (has)* causes severe defects in cardiac morphogenesis in which tilting of the heart cone is blocked and heart tube elongation is impaired. The *has* gene encodes Protein Kinase C iota (PRKCi) which is required for the establishment of apical-basal polarity of epithelial cells. PRKCs are components of the apical Par3 protein complex which has been linked to the apical Crumbs-Protein associated with Lin-seven 1 (Pals1)/MAGUK p55 subfamily member 5 (Mpp5) protein complex. Consistent with a function in apical-basal cell polarity, *has* mutants show defective formation and maintenance of several embryonic epithelia. During heart fusion and cone formation, myocardial cells are organized into two bilateral cell populations that have some characteristics of polarized epithelia. At these early stages, PRKCs are localized to the apical junctions of myocardial cells.

We have now shown that zebrafish Has/PRKCi is required tissue-autonomously within the myocardium for normal heart morphogenesis. We have also performed a functional analysis of Has/PRKCi by using a combination of antisense oligonucleotide morpholino mediated gene “knock down” and co-expression of mutant *has/prkci* mRNAs. This approach provides conclusive evidence that the catalytic activity of Has/PRKCi is essential within the context of cell polarity and heart morphogenesis. We conclude that phosphorylation targets of Has/PRKCi that are relevant for heart morphogenesis are present within the myocardium.

The zebrafish mutation *nagie oko (nok)* also causes severe epithelial defects similar to *has*. *nok* encodes a **m**embrane **a**ssociated **g**uanylate **k**inase (MAGUK) family protein with functional homology to mammalian Pals1/Mpp5. Using *nok/mpp5* antisense oligonucleotide morpholino mediated gene “knock down”, we have identified an early function of this gene in the polarized epithelial organization of myocardial cells prior to heart cone formation, whereby heart cone fusion is severely impaired. Moreover, zygotic *nok/mpp5* mutants have later myocardial defects including an incomplete heart tube elongation corresponding with a failure of myocardial cells to correctly expand in size. Together, these results demonstrate that cardiac morphogenesis depends on the polarized organization and coherence of the myocardium and that the expansion of myocardial cell size contributes to the transformation of the heart cone into an elongated tube.

Research in our laboratory is currently directed towards identifying and characterizing the direct downstream phosphorylation targets of Has/PRKCi in the context of cell polarity and organ morphogenesis. Furthermore, we are involved in the cloning and characterization of other zebrafish mutations that affect cellular polarity, epithelial integrity, and organ morphogenesis. The identification of the molecular pathways involved in vertebrate epithelial morphogenesis may lead to relevant animal models for human epithelial pathologies and to the development of novel therapeutic approaches.

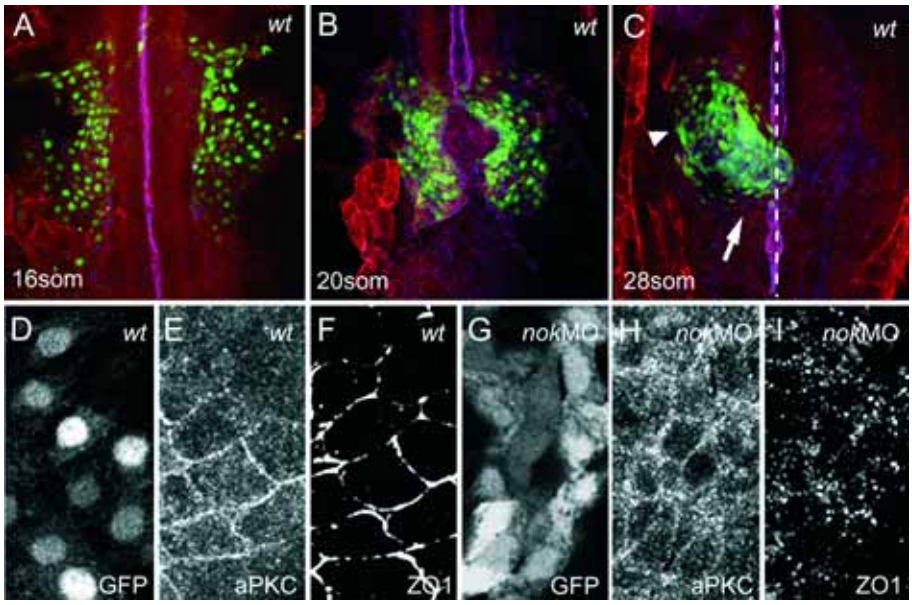

Figure 1

Zebrafish heart tube assembly is disrupted in *nok/mpp5* morphants. All images represent reconstructions of confocal Z-stack sections imaged on whole-mounts. *cardiac myosin light chain2*: GFP, green (nuclear GFP within myocardial cells); PRKC/aPKC, red; ZO-1, blue. PRKC and ZO-1 were used as a counterstain to visualize the embryonic midline (see also dotted line in D). **(A)** At the 16-somite stage, wildtype myocardial cells are organized as two bilateral sheets of cells. **(B)** Both sheets converge onto the midline where they fuse to form the heart cone around the 20-somite stage. **(C)** Heart cone tilting places the heart into the anterior-posterior orientation by the 28-somite stage. The atrium (arrowhead) is located to the left and anterior whereas the ventricle (arrow) is oriented towards the midline and posterior. **(D-I)** All images are details imaged from 16-somite stage whole-mounts. **(D-F)** At the 16-somite stage, wildtype myocardial cells have an epithelial organization with junctional distribution of PRKC and ZO-1. **(G-I)** At the 16-somite stage, *nok/mpp5* morphants, display a diffuse distribution of PRKC along membranes and ZO-1 junctional belts are disrupted and appear as spots. Orientation: Dorsal view and anterior to the top.

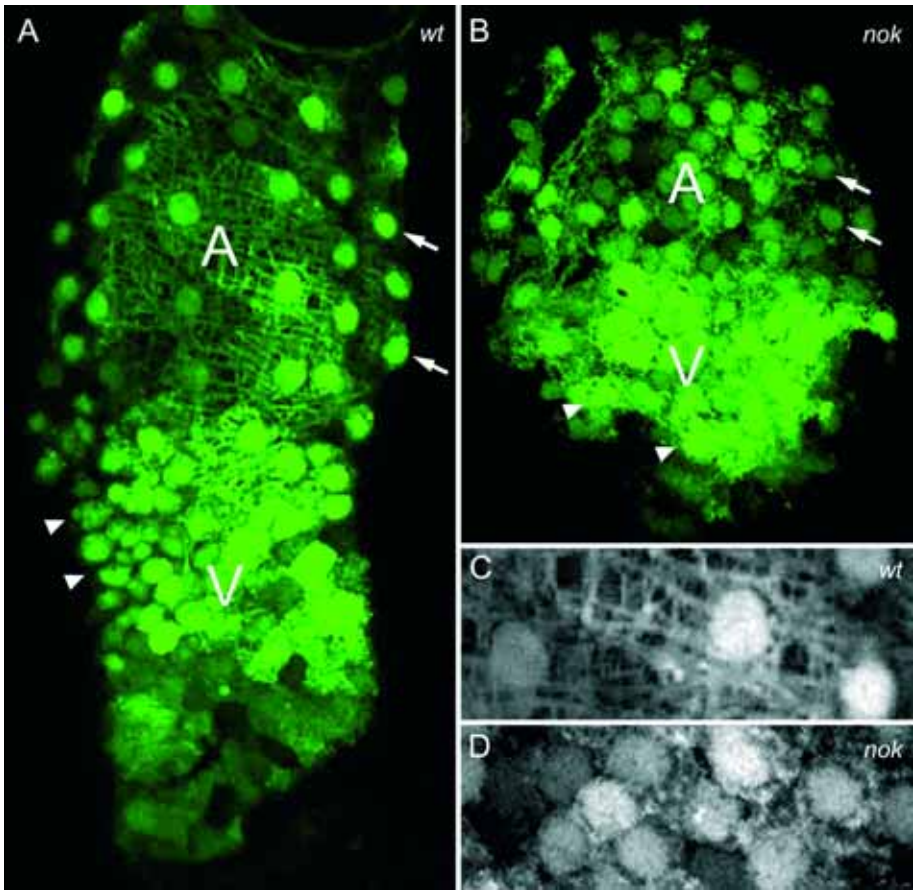

Figure 2

The zygotic function of *nok/mpp5* is required for myocardial cell remodeling. (A,C) 36 hpf *wt* and **(B,D)** *nok*^{s305} in the *Tg(cardiac myosin light chain2: GFP)* transgenic background. Images are reconstructions of confocal Z-stack sections. **(A,B)** Arrows indicate atrial myocardial and arrowheads ventricular myocardial cells. Shape and density of *nok*^{s305} mutant atrial and ventricular cells are similar. **(C,D)** Comparison of atrial myocardial cells in *wt* and *nok*^{s305} mutant hearts, indicating that *nok*^{s305} mutant atrial myocardial cells fail to expand in size and resemble ventricular myocardial cells. A, atrium; V, ventricle. Orientation: Dorsal view and anterior to the top.

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Molecular and Cell Biology of the (Epi)genome

M. Cristina Cardoso

Although the nucleus (from the Latin word 'nux') is the hallmark of eukaryotic cells, we still know remarkably little about its structure and function. For a long time, the nucleus has been underestimated as a mere repository of the genetic information packed into chromatin, freely floating like noodles in a soup of amorphous nucleoplasm. However, in the last decades, the development of antibodies to nuclear components combined with the ability to fluorescently tag proteins uncovered a different picture of the nucleus with many discrete and distinguishable subnuclear compartments involved in DNA or RNA metabolism. Unlike cytoplasmic organelles, subnuclear compartments are not limited by membranes raising the question as to how they arise and are maintained. Our goal is to understand how factors are assembled and recruited or excluded from their sites of action to control their biological function and, thereby, elucidate the principles and consequences of the dynamic nuclear organization. In particular, we focus on how the genetic and epigenetic information is replicated at every cell division and how it is "translated" during development into different gene expression programs defining the specific cell types and functions. Elucidation of mechanisms maintaining and changing the epigenome will lead to new approaches in disease prevention as well as therapy. In particular, epigenetic reprogramming may lend an entirely new perspective to regenerative medicine.

Dynamics of chromatin accessibility and proteome distribution in the nucleus

To elucidate the basic mechanisms responsible for this nuclear organization and its consequences, we are systematically probing the macromolecular features (size, charge, biochemical affinity) which determine the access to and interactions with different subnuclear compartments, such as transcriptionally active or inactive chromatin, the interchromatin space, and the nucleolus. To this end, we have developed and evaluated live-cell fluorescent markers for various subnuclear compartments and apply molecular and cell biology techniques in combination with quantitative live-cell microscopy methods, including fluorescence photobleaching, fluorescence correlation microscopy and single molecule tracking. In addition to established molecular techniques to introduce such molecular markers via DNA vectors, we are currently exploiting methods to introduce molecules directly via peptide transducing domains that can cross cellular membranes. We are now exploring their application in new molecular therapy strategies to improve heart function (in collaboration with I. Morano).

Dynamic organization of chromatin replication

DNA replication is the central event of the cell division cycle and is linked to cell cycle regulation and the cellular response to DNA damage in many ways. The precise and coordinated duplication of genetic information is critical for genome stability. Errors in DNA replication may trigger or promote cancer progression.

Replication of the mammalian genome starts at tens of thousands of origins that are activated at specific times during S phase raising the question as to how this replication program is coordinated. Importantly, the spatio-temporal progression of DNA replication is inherited through consecutive cell division cycles. We are studying the coordination of the multiple enzymatic activities involved in the replication of the genome preceding every mitotic division. With fluorescent fusion proteins and high resolution multidimensional time lapse microscopy, we could show that replication patterns within the nucleus change in a characteristic manner throughout S phase. Using biochemical *in situ* extractions and fluorescence photobleaching/activation techniques, we could show that the PCNA (proliferating cell nuclear antigen) clamp was tightly bound at replication sites, showing only little exchange over minutes, while other replication factors exchanged within seconds. These results suggest that the PCNA clamp forms a stable core bound to replicating DNA throughout the synthesis of several Okazaki fragments while other factors transiently associate with different kinetics. We are currently testing this hypothesis by simultaneously measuring the on/off rates of PCNA and PCNA-binding replication factors in living cells as well as by single molecule tracking.

A careful examination of the temporal and spatial assembly of new PCNA molecules by overlaying images collected at consecutive times indicated that PCNA assembles at adjacent sites, suggesting that activation of neighboring replication origins may occur by a "domino effect" possibly involving local changes in chromatin structure and accessibility. We are now trying to dissect the mechanisms that control the ordered activation of later replication origins and thus determine the replication program. Furthermore, we are analyzing links between DNA replication and repair, in particular the recruitment of DNA synthesis factors to DNA damage sites and the consequences for cell cycle progression and genome stability.

In addition, these studies have yielded a precise and direct way to identify in situ all cell cycle stages, which opens up new experimental approaches to study cell cycle-dependent protein dynamics in living cells.

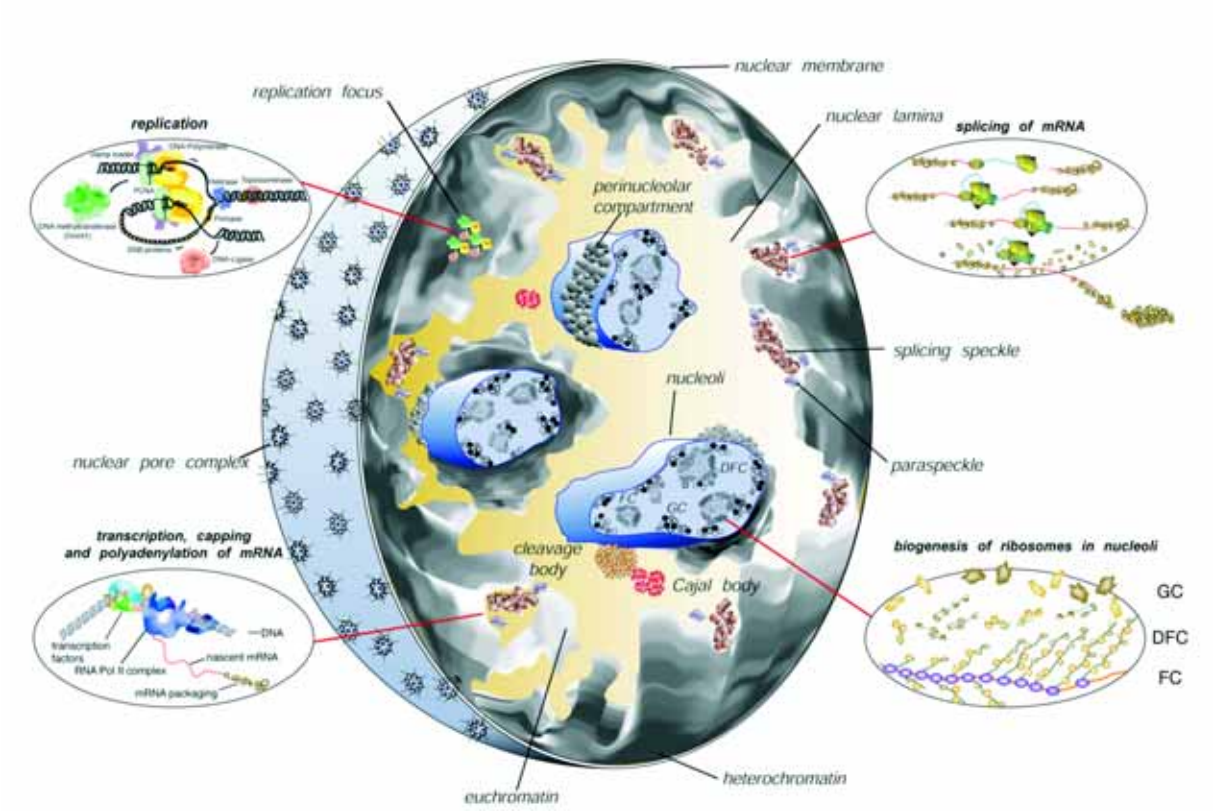

Figure 1
Law and order in the nucleus.
The graphic illustrates several of the different compartments identified in the nucleus with some of their functions highlighted and their relation to interphase chromatin organization.

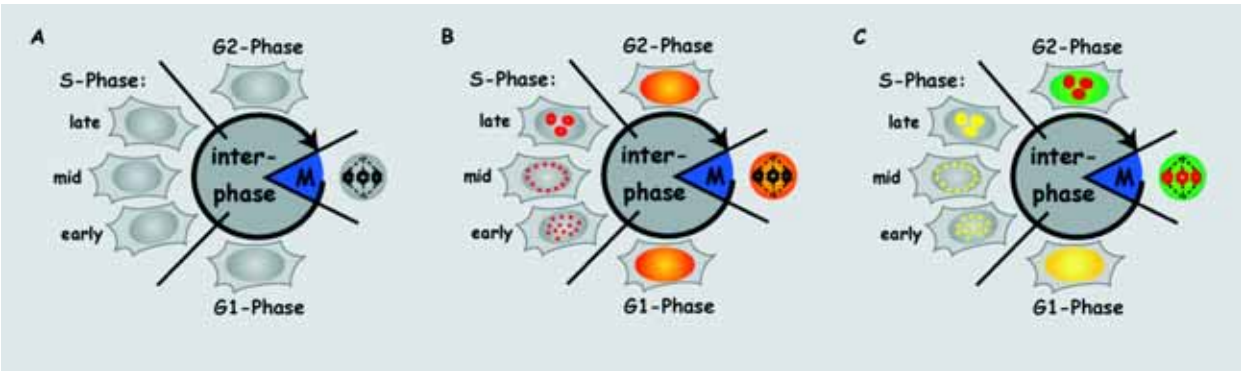

Figure 2
Cell cycle progression markers.
The graphic in A shows cells in different cell cycle stages where only the mitotic phase can be identified by its characteristic morphology. Visualization of fluorescent replication factors in B (e.g., DNA ligase I or PCNA) allows identification of the S phase and its progression. The combination with DNA methyltransferase I in C, further distinguishes the G1 and G2 phases.

Replication and translation of epigenetic information

Most cells of multicellular organisms contain identical genetic information but differ in their epigenetic information. The latter is encoded at the molecular level by post-replicative methylation of certain DNA bases (in mammals, 5-methyl cytosine at CpG sites) and multiple histone modifications. In addition, higher-order chromatin structures are established during cellular differentiation which impact genome expression and stability. As with the genetic information, the epigenetic information also needs to be duplicated and maintained over many cell generations. Our focus is on the role and regulation of DNA methylation. This epigenetic modification is essential for proper mammalian development and has been linked to human diseases (ie.g., cancer, Rett syndrome, and

Figure 3
Methyl CpG binding proteins induce clustering of heterochromatin during differentiation.
A 3D view through the nucleus (contour in red) of a living mouse myoblast cell (left) showing multiple heterochromatin centers (chromocenters, in green). Increasing the level of MeCP family members during terminal differentiation (myotubes, right) results in a large-scale reorganization and clustering of the highly methylated satellite DNA at chromocenters.

ICF syndrome). We are analyzing different proteins involved in the maintenance and change of this epigenetic modification and their dynamic interaction with the replication machinery and chromatin. Recently, we found that the main DNA methyltransferase Dnmt1 binds to the DNA replication machinery during S phase via its interaction with PCNA and binds to pericentric heterochromatin during G2 and M phase via a separate targeting sequence. This dual regulation might be required to reestablish the epigenetic information after DNA replication and to maintain stable gene expression patterns.

The epigenetic information needs to be "translated" to define specific cell types with specific sets of active and inactive genes, collectively called the epigenome. The methyl-cytosine binding proteins (MeCP2, MBD1-4) are involved in the translation of epigenetic information. These proteins are known to bind methylated cytosines and recruit other chromatin modifiers such as histone deacetylases. We have recently found that MBDs induce large-scale heterochromatin reorganization during terminal muscle differentiation. Based on this finding, we want to dissect the mechanisms responsible for this chromatin reorganization by a combination of *in vitro* and *in vivo* approaches including biochemical and photodynamic assays. This should help to elucidate the role of nuclear topology in cellular differentiation and provide new ways to manipulate the phenotypic plasticity of cells for application in cell replacement therapies in regenerative medicine.

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Immunology of Cardiovascular Diseases

Gerd Wallukat

The interest of our group is focussed on immunological processes in cardiovascular diseases. In several cardiovascular diseases, we discovered functional autoantibodies against extracellular structures of G-protein coupled receptors. We observed autoantibodies against adrenergic receptors and AT1-receptors in the sera of patients with myocarditis, dilated cardiomyopathy (DCM), peripartum cardiomyopathy, preeclampsia, vascular mediated kidney rejection, and essential and pulmonary hypertension. These autoantibodies recognize epitopes on the first or second extracellular loop of the receptors and act like the corresponding pharmacological agonists. In patients with myocarditis and dilated cardiomyopathy, but also in Chagas' disease, the autoantibodies recognize the β_1-adrenoceptor and, in some patients, the muscarinic M2 receptor as well. In recent years, we have investigated in more detail the effects of these autoantibodies. We believe that the antibodies stabilize the agonistic dimeric confirmation of the receptors resulting in the agonist-like effect.

Autoantibodies in myocarditis, dilated cardiomyopathy (DCM), and peripartum cardiomyopathy (PPCM)

The suggestion that the anti-β1-adrenoceptor autoantibody may play a role in the pathogenesis of DCM is supported by similar findings in patients with myocarditis, a disease widely held to be a precursor of DCM. We observed in patients with myocarditis that these autoantibodies disappeared spontaneously during the process of healing. In parallel, the left ventricular ejection fraction and heart rate were normalized. Moreover, it was shown that immunization of animals with the β_1-adrenoceptor or with peptides corresponding to this receptor caused a disease that corresponded to dilated cardiomyopathy.

Based on our autoimmune hypothesis, we proposed new therapeutic possibilities to treat patients with endstage dilated cardiomyopathy. One is unspecific immunoadsorption using columns that remove all IgG immunoglobulins from the patient's plasma. After this treatment, a marked improvement in cardiac function and normalization of the cardiac size were observed. The strong correlation observed between the reduction in the number of circulating autoantibodies to the β_1-adrenoceptor and the improvement of heart function support the hypothesis that the anti-β_1-adrenoceptor antibodies may play a role in the pathophysiology of myocarditis and DCM.

To confirm this hypothesis, we developed a specific immunoadsorption column. Based on our epitope analysis, a peptide column was generated that selectively removes the anti-β_1-adrenoceptor autoantibodies. In a pilot study, it was shown that the treatment of DCM patients with this specific adsorption improves cardiac function.

Similar antibodies against the β1-adrenoceptor were also observed in patients with peripartum cardiomyophathy (PPCM). This disease occurs 1 to 5 months after delivery and is very rare in Europe. However, the prevalence is high in the black population of South Africa (1:1000). In South Africa, 25% of the women with PPCM died. In this disease, the AAB recognize exclusively an epitope on the second extracellular loop of the β_1-adrenoceptor. The epitope is localized near the N-terminal part of this loop. Recently, we treated a patient with PPCM with immunoadsorption and observed a rapid improvement of the cardiac function after the removal of the β_1-adrenoceptor autoantibodies.

Autoantibodies in essential, refractory, and pulmonary hypertension

Furthermore, we have investigated the role of autoantibodies in essential, in therapy refractory, and in pulmonary hypertension. In the sera of patients with this disease, we detected autoantibodies directed against the α1-adrenoceptor. These autoantibodies recognize epitopes on the first or second extracellular loop of the α1-adrenergic receptor and act like α1-adrenergic agonists. In patients with refractory hypertension, more than 80% were antibody positive. In patients with malignant hypertension, we observed autoantibodies against the angiotensin II AT1-receptor.

Autoantibodies in preeclampsia and acute vascular kidney rejection

In both diseases, we detected autoantibodies against the angiotensin II AT1 receptor. In patients with preeclampsia, this IgG3 subclass antibody recognizes one epitope on the second extracellular loop. These agonist-like anti-AT1-receptor antibodies induce the formation of the transcriptions factors AP-1 and NFκB and activate NADPH oxidase. It appears that the antibodies were induced in the early phase of pregnancy by disturbances of the placental perfusion. The antibodies may play a role in elevating vascular resistance and promoting hypertension in these patients.

The AT1-receptor antibodies found in patients with acute vascular kidney rejection after transplantation bind to two different epitopes on the second extracellular loop. These antibodies are antibodies of the IgG1 and IgG3 subclass. The

agonistic effect of these antibodies were blocked by AT1-receptor antagonists and neutralized by peptides corresponding to the second extracellular loop of the AT1-receptor. As these antibodies activate –(beside their functional effect) transcription factors and the tissue factor, we assumed that they may be involved in the vascular mediated rejection of the kidney after transplantation. Based on our observation, we developed a new therapy which successfully prevents the allograft rejection.

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Neuromuscular and Cardiovascular Cell Biology

Michael Gotthardt

Introduction

Titin is a protein with multiple elastic and signaling functions derived from a complex subdomain structure. In striated muscle, it forms a continuous filament system and serves as a template to assemble the sarcomere providing multiple binding sites (see figure).

Titin is relevant in human disease, not only for its role in late stage cardiomyopathies, where changes in titin-isoform expression impair tissue elasticity, but also as the primary defect in various cardiac as well as skeletal muscle diseases. Currently, it is not known why the titin mutations identified so far cause either cardiac diseases or skeletal muscular dystrophy, although the mutated titin segments are expressed in all striated muscles.

Our long-term goal is to establish the role of titin in muscle and non-muscle function and disease, with the main emphasis on signal transduction and biomechanics. We have established the structure of the mouse titin gene and used gene targeting to create a set of knockout mice, which can be induced to express various mutant titin molecules lacking critical subdomains. With these mice, we have demonstrated that titin is crucial for embryonic development, assembly of the sarcomere, and muscle function.

Mechanotransduction

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 Tcap/telethonin and MLP protein. However, so far it is unclear as to 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 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.

Smooth muscle and non-muscle titins

Only recently, the muscle protein titin has been proposed to perform non-muscle functions, following its localization to various cell 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 stress fibers/cleavage furrows and in chromosome condensation through localization to mitotic chromosomes. Drosophila melanogaster deficient in the titin homologue D-titin show chromosome undercondensation, premature sister chromatid separation, and aneuploidy.

Our preliminary data indicate that titin is present in virtually every cell-type tested, albeit at lower levels than in striated muscle. Nevertheless, our knockout of titin's M-line 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 to vascular smooth muscle (elastic recoil of the aorta or "Windkessel Effect").

Functional analysis of individual titin domains

To lay the groundwork for the *in vivo* analysis of titin's multiple signaling, elastic, and adaptor domains, we have generated various titin mutant 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.

Understanding structural and biomechanical, as well as signaling and metabolic functions of titin, will help elucidate the pathomechanism of various cardiovascular diseases and, ultimately, aid the development of suitable therapeutic strategies.

Selected Publications

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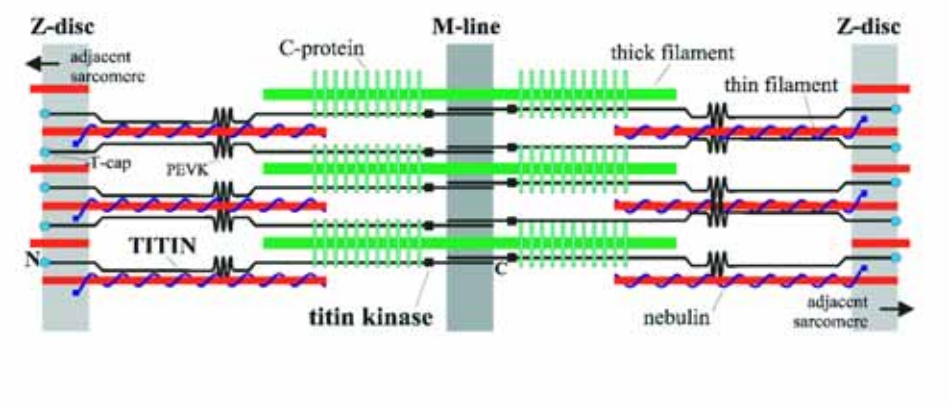
Witt, C.C., Ono. Y., Puschmann, E., McNabb, M., Wu, Y., Gotthardt, M., Witt, S.H., Haak, M., Labeit, D., Gregorio, C.C., Sorimachi, H., Granzier, H., Labeit, S. (2004) Induction and myofibrillar targeting of CARP, and suppression of the Nkx2.5 pathway in the MDM mouse with impaired titin-based signaling. J Mol Biol. 336(1), 145-54.



Schematic diagram of the sarcomere (modified from Gregorio et al., Curr. Opin. Cell. Biol. 11: 18-25, 1999). Titin forms a continuous filament system along the muscle fiber in vertebrate striated muscle overlapping in the M-line (titin C-terminus) and in the Z-disc (N-terminus). The titin kinase is found near the edge of the M-line 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.

Boucher, P.#, Gotthardt, M. #, Li, W.P., Anderson, R.G., Herz, J. (2003). LRP: Role in Vascular Wall Integrity and Protection from Atherosclerosis. Science 300, 329-32 (# = equal contribution).

Gotthardt, M., Hammer, H.E., Hübner, N., Monti, J., Witt, C.C., McNabb, M.M., Richardson, J., Granzier, H., Labeit, S., Herz, J. (2003). Conditional expression of mutant M-line titins results in cardiomyopathy with altered sarcomere structure. J. Biol. Chem. 278, 6059-65

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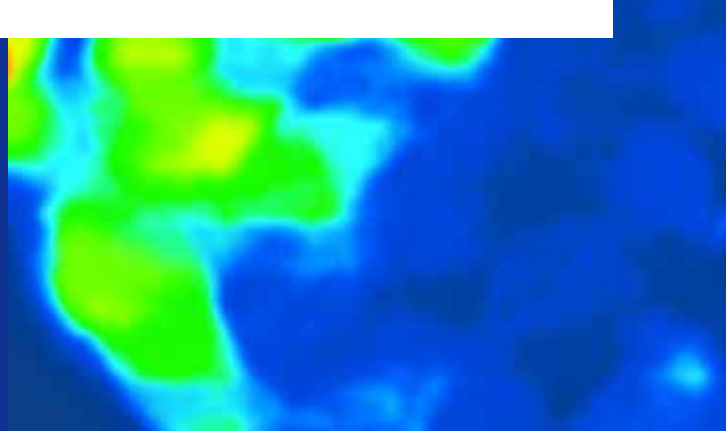

Bioinformatics

Jens Reich

The lipid network group continued its long-term project, which is a study, in collaboration with the Franz-Volhard-Clinic (Prof. F. Luft) and the MDC Genotyping Center (P. Nürnberg, now headed by N. Hübner), aiming at the dissection of the genetic factors that contribute to the cholesterol-containing blood lipoproteins LDL and HDL and to the concentration of triglycerides. All these metabolites are indicators of metabolic risk, in particular of developing arteriosclerosis and hyperlipidemia, which can be followed by myocardial infarction and cerebral stroke. It has been shown by previous studies that the relative contribution of genetic factors (so-called "heritability") to the risk or protection status of an individual is higher than 30% in most populations and conditions, in some circumstances (when the complementary non-genetic factors, i.e. lifestyle and eating habits) even 50% and higher. Such a value shows that a trait is to a similar degree caused by genetic and non-genetic factors. Since the latter are difficult to standardize and vary even between individuals and even within families, the dissection of the genetic factor is subject to a large statistical scatter due to the non-genetic confounding factors.

A risk status is clinically defined by a high LDL and a low HDL value, whereas a relatively protected metabolic status is characterized inversely by high HDL and low LDL. A genetic component of a trait in the population is usually revealed by statistical evaluation of family studies. Such high genetic influence is caused by variants in the DNA of lipid genes, which are transmitted from parents to children according to the rules of Mendelian genetics. Variation in the lipid status, insofar as being caused by inheritance, can be located in the relevant gene sequences, since the most widespread genetic variants on the population levels are single-nucleotide variants (acronym SNPs). Therefore, the specific task of the project is to find SNPs that segregate in families, to study their statistical properties, and to associate their occurrence in an individual with its lipid status. From general biochemical and physiological knowledge, one could predict that about 10 to 20 genes contribute collectively to the individual lipid status. Approximately every 10,000 base pairs occurs such a SNP (frequency > 3%) in most genes, so the average gene locus contains about 5-10 loci. It is necessary to study not only isolated SNPs but also their allelic association on individual chromosomes (so-called "SNP haplotypes"). The team of Klaus Rohde (also in the bioinformatics group) has long-standing theoretical and practical experience for this analytic task.

Preliminary theoretical estimates predicted that a population-representative sample of about 250 extended families (parents, several children, and perhaps relatives) with a total of about 1,200 subjects would be required in order to find the gene loci responsible for the lipid status. Such a "sample" was patiently recruited over many years by the Franz-Volhard group. They also measure the clinical status of the lipids. The genotyping center established the SNP status at 100 single nucleotide loci in 13 lipid-relevant genes. K. Rohde and his team then derived, by computer analysis, 230 haplotypes from the family distribution of SNPs.

We then performed variance component analysis, assuming a standard genetic model, of the influence of the 13 genes on the lipid status. The genetic variance on LDL explained 26%, on HDL explained 38%, and on LDL/HDL explained 28% of the total variance, respectively. The association of haplotypes in all the genes tested explained a major fraction of the genetic phenotypic variance component. For LDL, the association with haplotypes explained 67% and for HDL 58% of the genetic variance. We concluded that these haplotypes explain most of the genetic variance in LDL, HDL, and LDL/HDL in these representative German families. An analysis of the contribution to the genetic variance at each locus showed that *APOE (50%), CETP (28%), LIPC (9%), APOB (8%), and LDLR (5%) influenced variation in LDL. LIPC (53%), CETP (25%), ABCA1 (10%), LPL (6%),* and *LDLR (6%)* influenced the HDL variance. This study was the first extensive analysis of multiple haplotypes in multiple genes to predict the genetic variance on a complex trait.

Comparative study in independent population samples is a highly recommended, but rarely performed, methodological approach in genetic studies of complex traits. Its rationale is based on the assumption that a true association between varia-

tion at a candidate locus and variation of a phenotypic trait is likely to be confirmed across different population samples, while a spurious association will lead to discordant results. An opportunity to perform such a comparative study was provided by an epidemiology group at the University of Geneva (Switzerland). Nevertheless, unaware of the parallel Geneva study, our group had independently pursued a similar hypothesis, namely that common variants in multiple lipid-metabolism genes could predict lipid phenotypes. In Geneva, 1,708 French-speaking middle-aged adults were recruited and tested, while our group comprised 218 families with 1,054 individuals. These data were combined for replicate analysis. The results are at present being prepared for publication. We found that the genetic factors and the attribution to individual genes could be reproduced in both European populations. This confirmation is of high importance for the predictive value of SNPs for the lipid risk status of a person.

The team of K. Rohde is part of the Genetic-Epidemiological Methodological Centers (GEM) in the framework of the NGFN, and works in two NGFN-funded projects.

One project deals with the analysis of gene chip data for the 10K to 500K chips of Affymetrix, one of the most advanced and promising techniques for generating genome-wide SNP genotypes for population- and family-based studies, which allows coverage of the whole genome with a set of 10K, 100K, and, in the future, even more preselected SNP. This technology and its further development will form the basis of future genome-wide genotyping at the GMC. We plan in cooperation with the Cologne Center for Genomics (P. Nuernberg) to implement and establish a software basis for handling and analysing this large amount of genotypic data, to find linkage and association to genetic traits, and to use the technology for studying the genetic structure of the under-lying population. Already established in a program ALOHOMORA is the quality control and data conversion for linkage analysis. The program checks the gender of individuals and the correct family structure, identifies mendelian and non-mendelian errors (unlikely genotypes), and converts the data into the linkage format for Allegro, Genehunter, Merlin, and Simwalk2. Due to the constraints of linkage programs, the calculation can be performed optionally with sets of markers in the way of a moving window. Three genetic maps, DeCode, Marshfield, and SLM1 as well as allele frequencies of three ethnic populations, African-Americans, Asians, and Caucasians (provided by Affymetrix), can be selected. Further implementations will be interfaces to Mendel5.5 and association analysis programs. All data genotyped at the GMC shall be tested and compared for population stratification to characterize the underlying population.

The second project deals with the problem of linkage disequilibrium for multiple SNP markers along the genome and their association to complex genetic traits. Large-scale association studies have been proposed to be more promising for the identification of predisposing genes for complex diseases. Genome-wide linkage disequilibrium (LD) mapping of common disease could be more powerful than linkage analysis if the appropriate density of polymorphic markers were known and if the genotyping effort and costs were reduced. There is considerable evidence that discrete blocks of low diversity and high LD exist within the human genome, however their number is unknown. Within such blocks, information from some multiple single nucleotide polymorphisms (SNPs) may be redundant; the non-redundant subset of haplotype tagging SNPs (htSNPs) can distinguish the majority of haplotypes. Haplotypes capture the local LD information and are, therefore, more informative than single markers, because haplotypes contain more information on historical recombination and mutation events. The genotyping effort can potentially be reduced by estimating the optimal htSNPs using a small subsample of the study population that have been genotyped for a dense SNP map, so that genotyping of the whole sample is restricted to these htSNPs. With the decreased costs for individual genotyping, potentially larger study samples may be included, which should increase study power. Therefore, association studies using case-control designs with unrelated individuals or family controls may be more informative and cost-effective when based on haplotypes, whether to test a direct effect of a candidate gene or to fine-map an unknown gene by exploiting the pattern of LD. At present, work is in progress to estimate the number of stable LD blocks in the genome in their dependence on SNP marker choice and density using data of the HapMap project. On the basis of our research in the field of linkage disequilibrium and the introduction of an novel multilocus LD measure and the experience of assessing association of genetic traits to haplotype to multiple SNP genotypes of unknown phase by EM-algorithm and Markov Chain Monte Carlo technique, the aim of this project is to clarify some basic questions regarding association methods for mapping genes in complex diseases using SNPs, to incorporate results of these investigations into new approaches, and to give recommendations for the application of haplotype-based methods for genetic mapping of complex diseases.

Selected Publications

Knoblauch, H., Bauerfeind, A., Toliat, M.R., Becker, C., Luganskaja, T., Günther, U.P., Rohde, K., Schuster, H., Junghans,C., Luft, F.C., Nürnberg, P., and Reich, J.G. (2004). Haplotypes and SNPs in 13 lipid-relevant genes explain most of the genetic variance in high-density lipoprotein and low-density lipoprotein cholesterol. Hum. Mol. Genet. 13, 993-1004.

Pospisil, H., Herrmann, A., Bortfeldt, R.H., and Reich, J. (2004). EASED: Extended alternatively spliced EST database. Nucleic Acids Res. 32, D70-D74.

Rüschendorf, F, Nürnberg, P, (2005). ALOHOMORA: a tool for linkage analysis
using 10K array data. Bioinformatics 21, 2123-5.

Rohde, K, Fürst, R, (2003). Association of genetic traits to estimated haplotypes from SNP genotypes using EM algorithm and Markov Chain Monte Carle techniques. Hum. Hered. 56, 41-7.

Patent Applications

Use of haplotypes and SNPs in lipid-relevant genes fort the analysis and diagnosis of cardiovascular diseases.

Inventors:	Nürnberg, Reich, Luft, Knoblauch, Bauerfeind, Luganskaja, Rohde
MDC-Number:	MDC 0401 / EP
European Patent Registration:	04 003 654.3

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Medical Genomics and Gene Mapping Center

Norbert Hübner

Determining the molecular basis of natural phenotypic variation, including inter-individual susceptibility to common diseases, is a central challenge of post-genome genetics. Our group is investigating the molecular genetic basis of common cardiovascular risk factors and disorders in experimental rodent and human populations. Within the Gene Mapping Center (GMC) we are providing state-of-the art platforms for high throughput genotyping of single nucleotide polymorphisms (SNPs) and microsatellite markers for linkage and association mapping as well as genome wide expression profiling.

Genome approaches to dissecting cardiovascular and metabolic disease

Heritable differences in gene expression have been proposed to play critical roles in biomedical phenotype and evolution including inter-individual susceptibility to common diseases. The spontaneously hypertensive rat (SHR) is a widely studied model of human hypertension and also has many features of the metabolic syndrome. SHR and Brown Norway (BN) are founder strains for the BXH/HXB recombinant inbred (RI) strains, one of the largest rodent RI panels for analysis of cardiovascular and metabolic phenotypes. The reference BN genome sequence, together with availability of tens of thousands of rat SNPs, a dense genetic map and the previous mapping of over 70 physiological and pathophysiological phenotypes in this RI panel, make SHR an excellent model for dissection of complex cardiovascular and metabolic traits.

We applied integrated gene expression profiling and linkage analysis to the regulation of gene expression in fat, kidney, and adrenal tissue in the BXH/HXB panel of rat RI strains. About 15% of the eQTLs detected independently in the three tissues investigated were commonly regulated, with the majority acting in cis. This suggests that the preponderance of trans-acting eQTLs observed in the three separate tissues belong to tissue-specific networks for control of gene regulation. To investigate the overall effect of polymorphisms on gene expression levels, we compared the SNP frequency across the genome with the SNP frequency in eQTL genes. We found a highly significant enrichment of SNPs in the cis-regulated eQTL genes compared with either the trans-regulated eQTL genes or the observed rate across the genome. Cis-acting eQTLs are of particular interest as positional candidate genes for QTLs. We applied a comparative mapping strategy to explore the applicability of the detected cis-acting eQTLs to human hypertension. By forming a robust dataset of cis-acting eQTL genes with a false discovery rate <5% that were contained within rat blood pressure related QTLs, we identified the human orthologs and compared them with the location of previously mapped human blood pressure QTLs. This analysis defined a set of 73 attractive candidate genes for testing in human hypertension data sets.

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 seen in similar disorders in humans.

Gene Mapping Center (GMC)

The GMC serves as a core facility to MDC investigators. Moreover, it is open to any scientific collaboration that requires access to high throughput genotyping and expression technologies and assistance in the analysis of these datasets. These efforts are partially supported by the national genome research initiative, NGFN 2. Within NGFN 2, we are currently supporting a number of studies that encompass high throughput microsatellite marker and SNP genotyping projects for linkage analysis. In addition, we are providing genotyping, technical, and analytical support for a large-scale family based association study that aims to perform a genome wide association scan using half a million SNP genotypes per individual.

Selected Publications

Hubner, N, Wallace, CA, Zimdahl, H, Petretto, E, Schulz, H, Maciver, F, Müller, M, Hummel, O, Monti, J, Zidek, V, Musilova, A, Kren, V, Causton, H, Game, L, Born, G, Schmidt, S, Müller, A, Cook, SA, Kurtz, TW, Whittaker, J, Pravenec, M, Aitman, TJ. (2005). Integrated transcriptional profiling and linkage analysis for disease gene identification. Nature Genetics. 37, 243-253.

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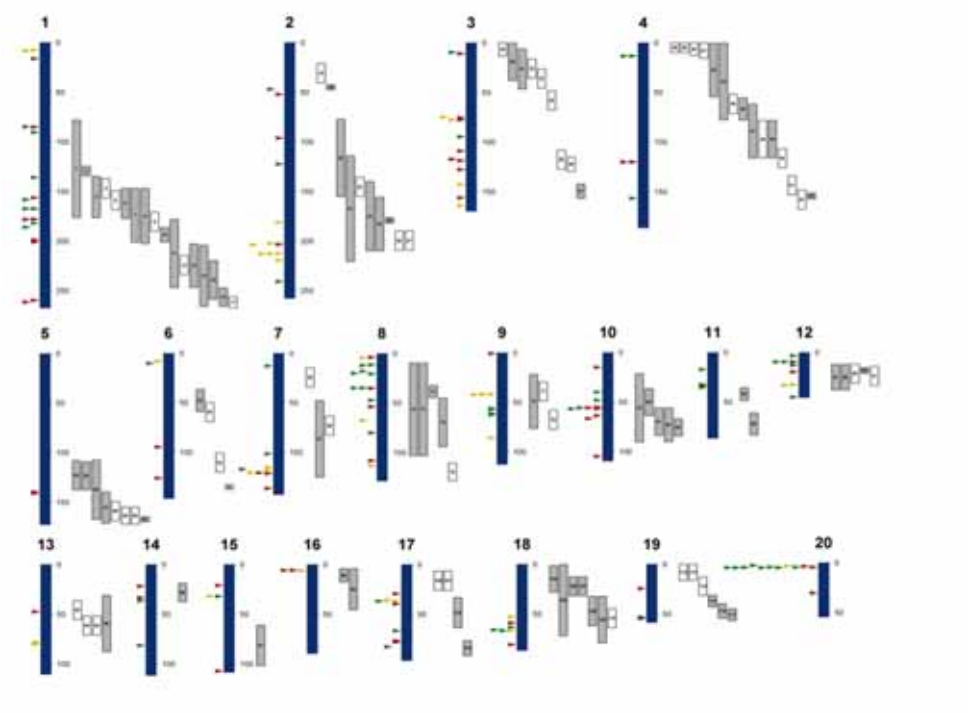
Rat Genome Sequencing Project Consortium. (2004). Genome Sequence of the Brown Norway rat yields insights into mammalian evolution. Nature. 428, 493-521.



Locations of cis-acting eQTLs and previously mapped SHR pQTLs. Chromosomes are shown in blue. The arrowheads on the left of each chromosome (yellow, fat; red, kidney; green, shared) represent the location of the probe set for each cis-acting eQTL with a false discovery rate < 5%. Previously identified pQTLs in SHR and the RI strains are shown on the right of each chromosome. Gray boxes represent pQTLs for which both flanking markers are mapped. White boxes represent pQTLs for which incomplete flanking marker information was available, in which case the flanking marker(s) are estimated to be 10Mbp from the linkage peak

Zimdahl, H, Nyakatura, G, Brandt, P, Schulz, H, Hummel, O, Fartmann, B, Brett, D, Droege, M, Monti, J, Lee, YA, Sun, Y, Zhao, S, Winter, E, Ponting, C, Chen, Y, Kasprzyk, A, Birney, E, Ganten, D, Hubner, N. (2004). A SNP map of the rat genome generated from transcribed sequences. Science. 303, 807.

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Genetics of Atopic Disease

Young-Ae Lee

The allergic diseases, particularly atopic dermatitis, food allergy, asthma, and hay fever, are among the most common chronic diseases in humans. 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. A strong genetic component in atopy and has been recognized. 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.

Clinical phenotyping

Genetic studies in complex human traits requires meticulous phenotyping of numerous patients and their families. Patient recruitment is performed at the Charité Children's Hospital. Atopic dermatitis in early childhood is an important risk factor for the development of asthma and hayfever. Most of the index children were below school age at the time of initial enrollment and may not yet have manifested allergic airways disease. The goal of the clinical phenotyping group is therefore to perform a prospective reevaluation of the families for atopic dermatitis and allergic airways disease by questionnaires, physical examination, and lung function testing. To obtain additional sub-phenotypes, a subset of families is being investigated for genome-wide gene expression levels from peripheral blood leukocytes.

Dissection of a locus for atopic dermatitis on chromosome 3q21

We have previously mapped a major susceptibility locus for atopic dermatitis to chromosome 3q21. Strong functional candidate genes within the 90% support interval of the AD locus were investigated for association with the disease phenotypes. CD80 and CD86 are type 1 membrane proteins of the immunoglobin superfamily that mediate important costimulatory signals for T cell activation and have been implicated in the activation of the Th2 subset of CD4+ T helper lymphocytes that play a pivotal role in mediating allergic inflammation. Genetic polymorphisms were evaluated for association with atopic dermatitis and atopy and no association with AD or atopy was detected.

Subregions on chromosome 3q21 were prioritized according to gene content for SNP identification and genotyping. In view of the overlapping linkage findings for the chronic inflammatory skin diseases atopic dermatitis and psoriasis, a 1,2 Mb subregion surrounding the SLC12A8 gene was targeted that had been shown to be linked and associated with psoriasis in a Swedish cohort. Microsatellite markers and SNPs in the region were examined for association with atopic dermatitis and atopy. No significant association was detected. We conclude that on chromosome 3q21 distinct genetic determinants for atopic dermatitis and psoriasis are operative and that the overlap of linkage findings may reflect close linkage of functionally related genes.

We have used the positional cloning approach to identify the susceptibility gene for atopic dermatitis on chromosome 3q21. To obtain higher resolution in the candidate region, linkage disequilibrium mapping has been performed. Genetic markers have been identified by sequence analysis and have been selected for genotyping. The family cohorts recruited in the NGFN during the first funding period have been used to replicate positive association findings.

Evaluation of GPRA in atopic dermatitis

The common genetic background of the allergic diseases is reflected by the atopic march in which a susceptible child passes a characteristic sequence of transient or persistent disease stages that begin with atopic dermatitis in the young infant and continue with the development of asthma and allergic rhinitis later in life. The close familial and intra-individual association of these disease entities strongly suggests shared genetic determinants. Recently, the gene encoding G protein-coupled receptor for asthma susceptibility (GPRA) was shown to be involved in the pathogenesis of atopy and asthma. The expression pattern of the disease associated B isoform, including tissues commonly associated with allergic reactions, such as skin keratinocytes, bronchial smooth muscle cells, bronchial epithelium, and intestinal epithelium, render the GPRA gene a prime candidate for the joint genetic origin of allergic disease. We have therefore evaluated the GPRA gene as a genetic risk factor for atopic dermatitis.

Using two large European family cohorts, including 826 children with atopic dermatitis, we conducted a family-based association test to avoid potential sources of error due to population admixture or stratification. No association with atopic dermatitis was detected. We conclude that the GPRA risk-haplotypes for asthma do not play a major role in the development of atopic dermatitis and that the GPRA gene does not contribute to the shared genetic predisposition of atopic dermatitis and allergic airways disease in our study population.

Selected Publications

Söderhäll C, Marenholz I, Nickel R, Grüber C, Kehrt R, Griffioen RW, Meglio P, Tarani L, Gustafsson D, Hoffmann U, Gerstner B, Müller S, Wahn U, Lee YA. Lack of association of the GPRA (G protein-coupled receptor for asthma susceptibility) gene with atopic dermatitis. J All Clin Immunol 2005;116(1):220-1.

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Lee, Y.A., Wahn, U., Kehrt, R., Tarani, L., Businco, L., Gustafsson, D., Andersson, F., Oranje, A.P., Wolkertstorfer, A., Berg, A., Hoffmann, U., Kuster, W., Wienker, T., Ruschendorf, F., Reis, A. (2000) A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. Nature Genet 26: 470-473.

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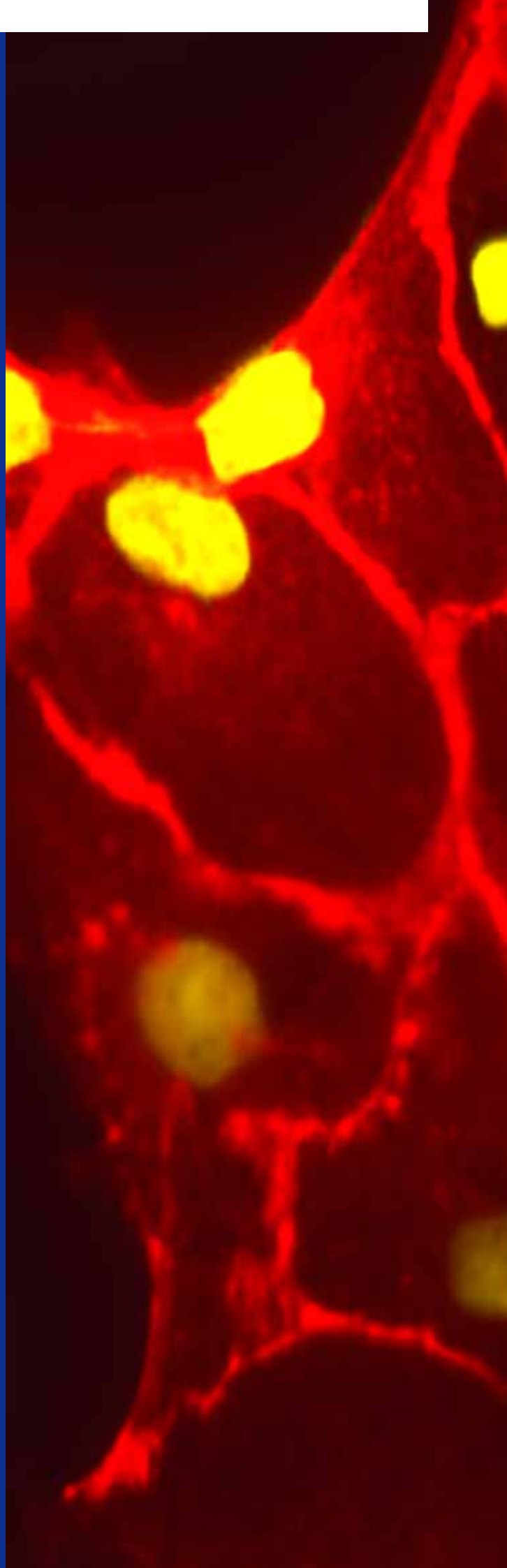
Cancer Research



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Signalling Pathways, Cell Biology, and Cancer
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Structural and Functional Genomics
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Cancer Research Program

Walter Birchmeier
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Cancer is a collective term of heterogeneous diseases that arise in different organs by mutations of the genome. The unifying premise of cancer cells is that they escape the natural "neighborhood watch" of growth control: cancer cells divide in an uncontrolled fashion, they escape natural cell death and the control of the immune system, and settle and grow elsewhere in the body by migrating through the blood- and lymph system (metastasis). The objective of the MDC Cancer Research Program is to understand how cancer develops and progresses and to use that knowledge to improve the diagnosis and, ultimately, the treatment of cancer.

How does cancer arise? The human genome consists of between 30,000 to 40,000 genes. Some of these genes are of particular importance in the regulation of cellular behavior. Many of these crucial genes are recurring targets of genetic alterations that provoke the emergence of cancer. These genes are categorized as either "oncogenes" or "tumor suppressor genes" and code for regulatory and structural proteins that control cell proliferation, differentiation, apoptosis, and cell migration. Many of them are also active during embryogenesis or during the development of distinct cell types. This is why tumor cells appear to share characteristics of embryonic cells.

The MDC Cancer Research Program consists of several scientific research groups that work in the fields of signal transduction and growth control, structural genome research, and tumor immunology. Knowledge in various basic biomedical and clinical disciplines is pooled to investigate the causes and the emergence of cancer and to find rational treatments. Cancer studies are conducted in close collaboration with clinically orientated groups at the Robert-Roessle Cancer Clinic of the Charité – Medical Faculty Berlin and at the HELIOS Clinic. The aim of the Cancer Research Program is to discover and to characterize genes that are responsible for the emergence of cancer and to determine how these gene products function in the above-mentioned crucial cellular processes and during progression of the disease. The resulting knowledge lays the essential groundwork for the development of future cancer treatments.

Krebsforschungsprogramm

Walter Birchmeier
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Martin Lipp
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Krebs ist ein Sammelbegriff für heterogene Erkrankungen malignen Zellwachstums, die durch Mutationen des Genoms entstehen können. Die Vorgeschichte von Krebszellen ist ähnlich: Krebszellen entziehen sich der natürlichen „Überwachung" durch Nachbarzellen. Sie teilen sich unkontrolliert, werden unsterblich, schirmen sich von der Kontrolle des Immunsystems ab, wandern über das Blut- und Lymphsystem und siedeln an verschiedenen Körperstellen, um Tochtergeschwulste (Metastasen) zu bilden. Das Ziel des MDC-Krebsforschungsprogramms besteht darin zu verstehen, wie Krebs sich entwickelt und fortschreitet, um diese Kenntnisse zur Verbesserung der Diagnose und auch zur Behandlung von Krebs anwenden zu können.

Wie entsteht Krebs? Das menschliche Genom besteht aus 30.000 bis 40.000 Genen. Einige dieser Gene sind für die Regulation des Zellverhaltens von besonderer Bedeutung. Viele dieser Schlüsselgene sind wiederkehrende Ziele genetischer Veränderungen, die die Entstehung von Krebs hervorrufen. Diese Gene werden als „Onkogene" oder „Tumorsuppressor"-Gene kategorisiert und kodieren Proteine, die die Zellteilung, -differenzierung, -apoptose und -wanderung kontrollieren. Viele dieser Gene sind auch während der Embryogenese oder der Entwicklung bestimmter Zelltypen aktiv.

Das Krebsforschungsprogramm des MDC setzt sich aus mehreren wissenschaftlichen Gruppen zusammen, die auf den Gebieten der Signalumwandlung und Wachstumskontrolle, der Strukturgenom-Forschung und der Tumorimmunologie tätig sind. Diese Untersuchungen werden in enger Zusammenarbeit mit den klinisch orientierten Gruppen der Robert-Rössle-Krebsklinik der Charité-Universitätsmedizin Berlin und der HELIOS-Klinik durchgeführt. Das Ziel des Krebsforschungsprogramms des MDC besteht darin, Gene zu entdecken und zu charakterisieren, die für das Entstehen von Krebs verantwortlich sind und zu ermitteln, welche Rolle diese Genprodukte in den o. g. entscheidenden Zellprozessen sowie im weiteren Verlauf der Krebserkrankung spielen. Das hieraus resultierende Wissen bildet den Grundstein für die Entwicklung zukünftiger Krebsbehandlungen.

Signalling Pathways, Cell Biology, and Cancer

NFκB is a pleiotropic regulator of gene expression involved in immune response, cell proliferation, and stress response. Normally, NFκB functions are held in check by inhibitory molecules, however, in Hodgkin disease NFκB is constitutively activated, as found earlier by Claus Scheidereit and Bernd Dörken. Both researchers have continued to explore the impact of dysregulated NFκB in Hodgkin Lymphoma and have now discovered a gene expression signature that reveals a plethora of NFκB target genes, all involved in advancing the growth of Reed-Sternberg tumor cells in Hodgkin disease. Identification of NFκB as one of the major gene regulators in Hodgkin disease immediately suggests inhibitors of the NFκB activation pathways as potential therapeutic reagents. Claus Scheidereit and Bernd Dörken were awarded the prestigious German Cancer Price in 2005 for their seminal work on Hodgkin disease achieved by a close collaboration between basic and clinical science and thus enabling a more rapid development of novel therapeutic approaches.

Two remarkable interdisciplinary scientific collaborations between Cancer Research and Cardiovascular Research at the MDC have yielded unexpected and far reaching results with respect to the understanding of and the therapeutic intervention in pathological processes. Walter Birchmeier's group has experimentally eliminated Plakophilin-2 in the mouse, a relative of β-catenin that plays a major causative role in human carcinoma. Plakophilin-2 is the binding partner of desmosomal cadherins and intermediate filaments at the cell surface in cell junctions (desmosomes). Drastic effects of Plakophilin-2 deletion on heart development in mice were observed. Now, Ludwig Thierfelder (research cardiologist at the MDC-associated Franz-Volhard-Clinic/Helios/Charité University Medical Center) and his group have examined patients with a specific heart disease called Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) for mutations in the Plakophilin-2 gene. The researchers were able to show that 25 – 30% of the ARVC patients have mutations in Plakophilin-2, linking for the first time a highly mutational-hot spot in a structural gene to cardiovascular disease. Asymptomatic mutation carriers can now be identified through genetic screening and treated prophylactically by implantation of a defibrillator to prevent heart attacks. In 2005, Walter Birchmeier was elected member of the renowned European Molecular Biology Organization (EMBO) for his work.

In addition to lymphomagenesis, NFκB has now also been implicated in hypertension. A problematic complication in hypertension is the thickening of the heart due to an increased work load by pumping blood through the body. Claus Scheidereit and Ruth Schmidt-Ullrich together with Martin Bergmann from the Franz-Volhard-Clinic were able to show that NFκB signaling is an essential trigger of heart hypertrophy, opening the possibility of preventing heart hypertrophy by blocking NFκB activity. Both examples (i.e. Plakophilin mutations and NFκB action in heart disease) represent major advances in cardiovascular research as a direct result of interdisciplinary collaboration between cell biologists and clinicians in cancer and cardiovascular research.

Signalwege, Zellbiologie, und Krebs

NFκB ist ein pleiotrophischer Regulator der Gen-Expression, der eine entscheidende Rolle in der Zelldifferenzierung, der Immunantwort und der Reaktion auf Stressbedingungen spielt. Unter normalen Umständen wird die NFκB-Aktivität/Funktion durch die Wirkung inhibitorischer Moleküle kontrolliert. Von Claus Scheidereit und Bernd Dörken wurde jedoch bereits vor einiger Zeit eine kontinuierliche Aktivierung von NFκB im Hodgkin-Lymphom festgestellt. Die beiden Forscher haben nun eine Gen-Expressions Signatur entdeckt, die eine Fülle von NFκB-regulierten Zielgenen einschließt, welche am Wachstum von Reed-Sternberg-Tumorzellen im Hodgkin-Lymphom beteiligt sind. Die Identifikation NFκBs als zentraler Genregulator im Hodgkin-Lymphom eröffnet damit neue Möglichkeiten in der Chemotherapie dieser Krankheiten. In der Zukunft könnten Inhibitoren der NFκB Aktivierung als Therapeutika eingesetzt werden. Für ihre herausragende Arbeit am Hodgkin-Lymphom und die enge Zusammenarbeit zwischen Grundlagenforschung und klinischer Forschung, sowie für die Entwicklung der Grundlage neuer therapeutischer Ansätze, wurden Claus Scheidereit und Bernd Dörken mit dem Deutschen Krebspreis 2005 ausgezeichnet.

Mit zwei bemerkenswerten Kooperationen zwischen der Krebsforschung und Herz-Kreislauf Forschung am MDC wurden zukunftweisende Ergebnisse in Bezug auf therapeutische Ansätze erzielt. In der Arbeitsgruppe Walter Birchmeiers wurde das Plakophilin-2 Gen in der Maus eliminiert. Plakophilin-2 ist ein mit β-Catenin verwandtes Molekül, welches eine Hauptrolle in der Karzinogenese spielt. Plakophilin-2 ist der Bindungspartner von Desmosomalen Cadherinen und Intermediärer Filamente auf der Zelloberfläche an Zellverbindungen (Desmosomen). Es zeigte sich, dass die Deletion von Plakophilin-2 in Mäusen einen drastischen Effekt auf die Herzentwicklung hat. Aufgrund des Zusammenhangs zwischen Herzentwicklung und Plakophilin-2 Funktion untersuchten nun Ludwig Thierfelder (Kardiologe an der MDC-assoziierten Franz-Volhard-Klinik/HELIOS/Charité Medizinzentrum der Universität) und seine Gruppe daraufhin Patienten mit einem spezifischen Herzfehler, der Arrhythmogenen Rechts-Ventrikulären Kardiomyopathie (ARVC), auf Mutationen im Plakophilin-2-Gen. Der Forschungsgruppe gelang der Nachweis, dass zwischen 25-30% der ARVC-Patienten eine Mutation im Plakophilin-2 Gen tragen. Mit diesem Forschungsergebnis konnte zum ersten Mal ein Mutations „Hot-Spot", wie man solche oft in Tumoren findet, mit einer verbreiteten Herzkrankheit in Verbindung gebracht werden. Träger der Mutation, bei denen die Krankheit bislang symptomfrei verlaufen ist, können nunmehr durch genetisches Screening identifiziert werden und durch prophylaktische Implantation eines Defibrillators vor dem Herztod gerettet werden. 2005 wurde Walter Birchmeier für seine Arbeiten zum Mitglied der angesehenen EMBO-Organisation (EMBO-Member) gewählt.

Neben der Lymphomagenese spielt NFκB auch im Bluthochdruck eine Rolle. Eine der Hauptkomplikationen bei Bluthochdruck ist die Vergrößerung des Herzmuskels (Hypertrophie) als Folge seiner erhöhten Arbeitslast. Claus Scheidereit und Ruth Schmidt-Ullrich haben zusammen mit Martin Bergmann von der Franz-Volhard-Klinik herausgefunden, dass NFκB ein entscheidender Auslöser der Herz-Hyper-

Metastasis is the major pathological complication in solid tumors. Metastasis is often associated with an epithelial-mesenchymal transition that permits cancer cells to migrate elsewhere in the body to initiate secondary tumors. The laboratory of Walter Birchmeier has now discovered that the product of the BCL9 gene induces epithelial-mesenchymal transition by interfering with β-catenin binding to the cell membrane. In addition, BCL9 helps keeping β-catenin in the nucleus and increases the transcriptional activity of β-catenin.

Peter Schlag's research group has identified two proteins predictive for cancer and metastasis development which might function as potential future therapeutic targets. One protein, termed prognostin, appears to be predictive for colon cancer formation and is functionally involved in receptor tyrosine kinase signaling and enhanced tumor growth. A second protein, metastasin, was identified as a direct target of Wnt/β-catenin signalling. Metastasin is involved in cell migration and invasion, and might become an important marker of metastasis formation.

Cell division of normal and leukemic blood cells depends on the product of the Myb proto-oncogene. A mutant version of Myb was already isolated 70 years ago in a retrovirus that induces myeloblastosis in birds. It was later discovered that viral Myb is a recombined descendant of the normal cellular Myb counterpart. Cellular Myb acts as gene regulator balancing proliferation and differentiation in blood cells, a process disrupted in leukaemia. Now, researchers in the laboratory of Achim Leutz have shown that Myb assists in the modification of histone proteins by acetylation of lysine residues. This mechanism is crucial during chromatin remodelling and determines whether genes are expressed or remain silent. The normal Myb protein helps catalyze the acetylation of histones at genes involved in myeloid differentiation, whereas the leukemic Myb version has lost this ability and, thus, prevents cell differentiation. Achim Leutz was elected member of the prestigious European Molecular Biology Organization (EMBO) in 2005 for his work.

It has been previously demonstrated that cells have a cancer protection program termed cellular senescence which irrevocably arrests cells in G1 phase and stops them from further cell division. Now Clemens Schmitt and co-workers have shown in a landmark discovery that the anti-cancer senescence program prevents the ras-oncoprotein from induction of lymphoma. The mechanism they were able to discover involves an enzyme already known to shut down genes by altering the methylation status of their respective chromatin targets at lysine residues on histone tails. This senescence pathway works in concert with the apoptosis pathway and is also downstream of p53. In contrast to p53 mutations frequent in cancer development, mutations of the senescence pathway leave the cells responsive to therapeutic treatment capable of inducing apoptosis, however.

An intriguing feature of cancer cells is their dysregulated cell cycle which induces untimely cell division. Accurate replication of cellular DNA is a prerequisite for successful cell division. We know surprisingly little about how replication of nuclear DNA originates in higher eukaryotes although it is

trophie ist. Diese Erkenntnis eröffnet die Möglichkeit der Behandlung von Hypertrophie durch Blockieren NFκB-assoziierter Signaltransduktionswege. Beide Beispiele, Plakophilin und NFκB bei der Entstehung von Herz-Kreislauf-Krankheiten und von Krebs, sind von weitreichender Bedeutung und dokumentieren ein erfolgreiches, zukunftsweisendes Ergebnis der interdisziplinären Forschung zwischen Zellbiologen und Klinikern am MDC.

Die Metastasierung von malignen Tumoren ist eines der Hauptprobleme bei der Behandlung von Krebs. Der Prozess der Metastasierung geht häufig mit einer epithelial zu mesenchymalen Umwandlung der Krebszellen einher, in deren Folge sich Krebszellen im Körper verbreiten und die Bildung neuer Tochtertumore initiieren können. In der Gruppe Walter Birchmeiers wurde nun herausgefunden, dass das Produkt des BCL9-Gens den mesenchymalen Übergang fördert, indem es die Bindung β-Catenins an die Zellmembran verhindert. Zudem hält das BCL9-Genprodukt β-Catenin im Zellkern fest und führt so zu einer Verstärkung der β-Catenin-vermittelten Genaktivierung.

Auf der Suche nach neuartigen zellulären Zielstrukturen zur Diagnose und Behandlung metastasierender Tumore konnten in der Arbeitsgruppe Peter Schlags zwei Proteine charakterisiert werden: Prognostin und Metastasin. Prognostin ist möglicherweise ein Marker zur Erkennung einer Kolonkarzinom-Entstehung, spielt eine Rolle in der Signalübertragung durch Rezeptor-Tyrosin-Kinasen und fördert das Tumorwachstum. Das weitere Protein, Metastasin, wurde als direktes Zielgen des Wnt/β-Catenin-Signal-Transduktionweges identifiziert. Das Metastasin-Protein ist an der Zellmigration und -invasion beteiligt und könnte sich als wichtiger Marker für die Entstehung von Metastasen erweisen.

Die Zellvermehrung von normalen als auch von leukämischen Blutzellen wird durch das Produkt des c-Myb-Gens gesteuert. Eine mutierte Version von Myb wurde bereits vor 70 Jahren in einem Retrovirus entdeckt, das in Vögeln Myeloblastosen hervorruft. Später wurde auch das zelluläre Gegenstück von Myb entdeckt, welches durch einen Rekombinationsvorgang in mutierter Form in das Virus integrierte und nun Leukämie verursacht. Zelluläres Myb agiert als Transkriptionsfaktor und stellt die Balance zwischen Zellproliferation und Differenzierung ein. Dieser Prozess ist in Leukämien gestört. In der Arbeitsgruppe Achim Leutz' konnte nun gezeigt werden, dass Myb an der Modifikation eines Histon-Proteins durch Azetylierung beteiligt ist und somit die Struktur des Chromatins ändert. Dieser Mechanismus ist ein essenzieller Schritt bei der Genregulation, die von der Struktur des Chromatins abhängt. Das normale Myb-Protein unterstützt die Azetylierung von Histonen an Genen, die an der myeloiden Zelldifferenzierung mitwirken. Die leukämogene Myb-Version kann diesen Prozess nicht durchführen und verhindert dadurch die Zelldifferenzierung. Achim Leutz wurde 2005 für seine Arbeiten zum Mitglied der angesehenen EMBO-Organisation (EMBO-Member) gewählt.

Vor einigen Jahren wurde gezeigt, dass Zellen vor unkontrollierter Zellteilung durch Zellalterung (Seneszenz) geschützt sind. Dieses Programm der zellulären Alterung arretiert Zellen in der G1-Phase des Zellzyklus und verhindert dadurch

evident that many potential targets for future cancer therapies might be found in the machinery that regulates DNA replication. The group of Manfred Gossen has now made considerable progress in analyzing the protein complex required for initiating replication in vertebrate nuclear DNA.

The secretory pathway of eukaryotic cells harbors an elaborate protein quality control system, which prevents the accumulation of misfolded or unassembled proteins in the secretory pathway. This system is localized in the Endoplasmic Reticulum (ER). ER associated protein degradation (ERAD) is an important component of this quality assurance system and directs misfolded proteins for destruction by the cytoplasmic ubiquitin-proteasome pathway. The group of Thomas Sommer has now identified a new component, Ubx2/Sel1p, necessary for ERAD. In addition, the scientists have shown that Ubx2/Sel1p function is not limited to ERAD substrates but is also involved in breakdown of cytosolic substrates that are processed by membrane-bound ubiquitin ligases.

Evolution of the genomes is strongly affected by molecular parasites termed transposable elements. Transposable elements are mobile segments of DNA that are ubiquitous in most living organisms. In humans, approximately half of the genome consists of such or related elements. Transposable elements may excise and integrate at different locations, thus inducing DNA damage and dysregulating gene expression. DNA damage repair and gene silencing mechanisms appear to have evolved closely together in order to restrict transposon functions. The laboratories of Zoltan Ivics and Zsuzsanna Izsvak are working on unravelling the molecular mechanisms involved in transposition. Their research is aimed at elucidating how transposition is regulated in vertebrates, and by which molecular interactions host DNA repair mechanisms function to minimize DNA damage inflicted by transposons, a process which has enabled a peaceful coexistence during evolution. Their findings will contribute not only to the understanding of the molecular events leading to cancer by failure of DNA damage repair mechanisms, but also to the development of safer gene therapeutic vectors. Zsuzsanna Izsvak was awarded the “European Young Investigator Award”, which enabled her to establish her own independent research group with a prestigious and generous grant, thus intensifying transposon research at the MDC.

Further newcomers to the MDC are Daniel Besser, working on signalling in human embryonic stem cells, Ulrike Ziebold, working on mechanisms of tumor progression and metastasis, and Frank Rosenbauer, working on the concept of cancer stem cells and transcription factor function in hematopoietic and leukemic stem cells. Both Ulrike Ziebold (Marie Curie Excellence Grant, FP6) and Frank Rosenbauer (Helmholtz-University Young Investigators Group) have been able to raise extramural funding to establish independent research groups at the MDC. Moreover, Ulrike Ziebold has been awarded with the Monika Kutzner Prize by the Berlin-Brandenburg Academy of Science.

weitere Zellteilung. Clemens Schmitt hat zusammen mit seinen Mitarbeitern herausgefunden, dass das Ras-Onkoprotein durch das Anti-Tumor-Zellalterungprogramm an der Induktion von Lymphomen gehindert wird. Der von der Forschungsgruppe beschriebene molekulare Mechanismus basiert auf einem Enzym, das bereits durch seine Fähigkeit bekannt war, die Gen-Expression durch die Änderung des Methylierungszustandes von Histon-Proteinen zu ändern. Der Signalweg zur Zellalterung arbeitet in engem Zusammenspiel mit Apoptose-Signalwegen und wird zudem von dem Tumorsuppressor-Protein p53 reguliert. Im Gegensatz zu Tumoren, die Mutationen in p53 tragen, bleibt bei Tumoren mit Mutationen im Zellalterungssignalweg die Empfindlichkeit gegenüber Krebstherapien erhalten.

Eine bemerkenswerte Eigenschaft von Krebszellen ist ihr deregulierter Zellzyklus und damit die Eigenschaft der unkontrollierten Zellteilung. Voraussetzung für eine erfolgreiche Zellteilung ist die genaue Replikation der DNA. Obgleich die DNA-Replikationsmaschinerie wahrscheinlich eine Fülle geeigneter Zielstrukturen für neuartige Krebstherapien bereithält, ist über die molekularen Mechanismen eukaryontischer DNA-Replikation überraschend wenig bekannt. Manfred Gossen hat mit seiner Arbeitgruppe beachtliche Fortschritte in der Analyse von eukaryontischen Proteinkomplexen erzielt, die an der DNA-Replikation beteiligt sind.

Der Sekretionsweg eukaryontischer Zellen enthält ein effizientes Qualitätssicherungssystem, das die Akkumulation von falsch gefalteten oder nicht assemblierten Sekretproteinen verhindert. Dieses System ist im Endoplasmatischen Retikulum (ER) lokalisiert und funktionell an die ER-assoziierte Proteolyse (ERAD) gekoppelt. ERAD sorgt dafür, dass die als falsch erkannten Proteine zurück in das Zytosol transportiert werden und dort einem ubiquitin- und proteasomabhängigen Abbau unterliegen. Die Gruppe von Thomas Sommer konnte eine neue zentrale Komponente dieses ERAD Systems, Ubx2/Sel1p, identifizieren und charakterisieren. Die Funktion von Ubx2/Sel1p ist nicht nur auf ERAD beschränkt, sondern erstreckt sich auch auf den Abbau zytosolischer oder nukleärer Proteine die durch Ubiquitinligasen der ER-Membran prozessiert werden.

Die Evolution des Genoms wurde in großem Maße von so genannten Transposablen Elementen mitgestaltet. Beispielsweise besteht das menschliche Genom etwa zur Hälfte aus solchen oder ähnlichen Elementen. Transposons konnten bislang im Erbgut von Bakterien, Pflanzen und Vertebraten nachgewiesen werden. Sie haben die Eigenschaft, sich aus der DNA herauszuschneiden und sich später an anderer Stelle wieder in die DNA zu integrieren. Diese Vorgänge können zu DNA-Schäden und zur Fehlregulation der Gen-Expression führen. Um dies zu verhindern, haben sich zelluläre DNA-Reparaturmechanismen herausgebildet, die das eigenständige Agieren von Transposons unterdrücken. Die molekularen Mechanismen dieses Vorganges werden am MDC von Zoltán Ivics und Zsuzsanna Izsvák untersucht. Besonderes Augenmerk gilt ihnen dabei dem Wechselspiel der Erbgutschädigung durch Transposons und der DNA-Reparaturmaschinerie, die Schäden durch Transposons begrenzt. Neben einem besseren Verständnis der Transposonbiologie und ihrem Beitrag zur Krebsentwicklung, bietet die Forschung beider Wissenschaft-

ler eine Grundlage für die Entwicklung sicherer Vektoren für die Gentherapie. Als Anerkennung ihrer wichtigen wissenschaftlichen Arbeit wurde Zsuzsanna Izsvák der „European Young Investigator Award“ zuerkannt, der ihr die Einrichtung einer eigenen unabhängigen Forschungsgruppe zur Intensivierung der Transposonforschung am MDC ermöglichte.

Zu den Neuzugängen am MDC zählen auch Daniel Besser mit seiner Forschung an Signaltransduktionswegen in humanen embryonalen Stammzellen, Ulrike Ziebold mit ihrer Forschung an der Tumorprogression und Metastasierung, sowie Frank Rosenbauer, der das Konzept von Krebs-Stammzellen bei der Leukämieentstehung untersucht. Ulrike Ziebold (Marie Curie Excellence Grant der EU) und Frank Rosenbauer (Junior-Gruppe aus dem Fonds des Helmholtz-Präsidenten) haben sich zudem erfolgreich für Drittmittel zum Aufbau einer eigenen Arbeitsgruppe am MDC bewerben können. Außerdem ist Ulrike Ziebold mit dem Monika-Kutzner-Preis der Berlin-Brandenburgischen Akademie der Wissenschaften ausgezeichnet worden.

Structural and Functional Genomics

Structural studies of proteins and their interactions with other molecules benefit greatly from technical developments made recently within the context of structural genomics (structural proteomics) initiatives. In the laboratory of U. Heinemann, these techniques are used to characterize the structural basis of protein-DNA interaction, vesicular transport, and cellular processes related to human diseases. The plasmid RP4-encoded KorB protein, involved in gene regulation and plasmid partitioning, was shown to recognize its DNA target sequence in an unusual way where protein-DNA contacts made by a helix-turn-helix motif play a secondary role and sequence read-out is achieved by contacts outside this motif. In the area of vesicle-based intracellular transport, studies of the TRAPP tethering complex, localized to the Golgi membrane, have yielded the crystal structures of the palmitoylated subunit BET3 and a further subunit, TPC6. These two proteins were shown to form heterodimers, representing the first sub-complex of TRAPP identified so far.

Current treatment protocols for hepatitis B virus (HBV) infection are effective but hampered by the selection of drug resistant HBV strains. Within a research program aimed at developing new antiviral compounds against HBV, new L-nucleosides and their corresponding triphosphates, targeting the catalytic site of HBV DNA polymerase, were synthesized (E. Matthes). Between the newly synthesized compounds, β-L-2’,3’-didehydro-2’,3’-dideoxy-N4-hydroxycytid in (hydroxy-ddeC) and N4-hydroxy-thiacytidine (hydroxy-3TC) proved to be the most active in suppressing HBV replication. Surprisingly, hydroxy-3TC was completely inactive against HIV infections.

Human breast cancer is a very heterogeneous disease. The majority of sporadic breast cancers result from an accumulation of gene mutations in somatic cells. In addition to highly penetrant inherently predisposing mutations in at least two genes, BRCA1 and BRCA2, more genetic factors with variable penetrance in causing breast cancer are awaiting identifica-

Strukturelle und funktionelle Genomik

Strukturuntersuchungen an Proteinen und ihren Wechselwirkungen mit anderen Molekülen bedienen sich zunehmend methodischer Entwicklungen, die kürzlich im Rahmen von Initiativen der Strukturellen Genomik (Strukturellen Proteomik) stattfanden. Im Labor von Udo Heinemann werden diese Techniken eingesetzt, um die strukturelle Basis von Protein-DNA-Interaktionen, des vesikulären Transports und krankheitsbezogener zellulärer Prozesse aufzuklären. Für das vom Plasmid RP4 kodierte und in Genregulation und Plasmidpartitionierung involvierte Protein KorB konnte ein ungewöhnlicher Modus der DNA-Bindung aufgezeigt werden, in dem das „Helix-Turn-Helix“-Motiv des Proteins eine sekundäre Rolle spielt, wohingegen die Sequenzerkennung über Kontakte außerhalb dieses Motivs realisiert wird. Auf dem Gebiet des vesikelgestützten intrazellulären Transports haben Arbeiten am Golgi-lokalisierten Anheftungskomplex TRAPP zu Kristallstrukturen der palmitoylierten Untereinheit BET3 sowie einer weiteren Untereinheit, TPC6, geführt. Die Bildung von Heterodimeren aus diesen beiden Proteinen, die damit einen ersten identifizierten Sub-Komplex von TRAPP repräsentieren, konnte nachgewiesen werden.

Aktuelle medikamentöse Protokolle zur Behandlung von Infektionen mit dem Hepatitis B-Virus (HBV) sind wirksam, führen jedoch oft zur Selektion resistenter HBV-Stämme. Als Teil eines Forschungsprogramms zur Entwicklung gegen HBV gerichteter antiviraler Verbindungen wurden neue L-Nukleoside und die entsprechenden Triphosphate, die gegen das katalytische Zentrum der DNA-Polymerase von HBV gerichtet sind, synthetisiert (E. Matthes). Von den neu synthetisierten Substanzen bewirkten β-L-2’,3’-Didehydro-2’,3’-dideoxy-N4-hydroxycytidin (Hydroxy-ddeC) und N4-Hydroxy-thiacytidin (Hydroxy-3TC) die ausgeprägteste Suppression der Replikation von HBV. Überraschenderweise war Hydroxy-3TC gegen HIV-Infektionen völlig inaktiv.

tion. A search for genes and gene networks involved in breast cancer uses a combined functional approach of microcell mediated chromosome transfer and expression difference analysis (S. Scherneck). With this approach, γ-aminobutyric acid receptor-associated protein, a novel tumor suppressor showing reduced expression in breast cancer was characterized and ST18, located at human chromosome 8q11.2, was identified as a breast cancer tumor suppressor gene.

A comprehensive analysis of the sequence-dependent structure and dynamics of double-stranded DNA can only be achieved using computer simulation. As part of a large international research consortium, Heinz Sklenar has obtained conformational parameters for the 136 unique tetranucleotides occurring in DNA using Molecular Dynamics and Monte Carlo approaches. A computer modeling study of Methylene Blue binding to DNA has revealed both minor-groove binding and base-pair intercalation as possible modes of association. Modeling techniques developed in the laboratory have been used to reveal the structural and energetic origins of sequence-specific DNA binding by the papilloma virus E2 protein.

Brustkrebs ist eine sehr heterogene Erkrankung. Die Mehrheit der sporadischen Fälle von Brustkrebs ist das Ergebnis einer Akkumulation von Genmutationen in somatischen Zellen. Zusätzlich zu den bekannten Genen BRCA1 und BRCA2, deren Mutationen mit hoher Penetranz Brustkrebs auslösen, gilt es weitere, mit variabler Penetranz Brustkrebs auslösende Gene zu identifizieren. In einer Suche nach Genen und Gen-Netzwerken mit Bezug zur Entstehung von Brustkrebs wird ein kombinierter funktioneller Ansatz eingesetzt, der Mikrozellen-vermittelten Chromosomentransfer mit differenzieller Expressionsanalyse verbindet (S. Scherneck). Mit diesem Ansatz wurde das γ-Aminobuttersäurerezeptor-assoziierte Protein, ein neuer Tumorsuppressor mit reduzierter Expression in Brustkrebsgewebe, charakterisiert. Daneben wurde das auf dem menschlichen Chromosom 8q11.2 lokalisierte Gen ST18 als Brustkrebs-Tumorsuppressor-Gen identifiziert .

Eine umfassende Analyse der sequenzabhängigen Struktur und Dynamik doppelsträngiger DNA ist nur durch Computeranalyse möglich. Als Mitglied eines großen internationalen Forschungs-Konsortiums hat Heinz Sklenar Konformationsparameter für die 136 einzigartigen Tetranukleotide der DNA mittels Molecular-Dynamics- und Monte-Carlo-Ansätzen bestimmt. Die Computersimulation der Bindung von Methylenblau an DNA identifizierte sowohl Einlagerung in die kleine Furche, als auch Interkalation zwischen die Basenpaare als mögliche Modi der Assoziation. In der Arbeitsgruppe entwickelte Modellierungstechniken erlaubten schließlich die Aufklärung der strukturellen und energetischen Grundlagen der sequenzspezifischen DNA-Bindung des E2-Proteins des Papillomavirus.

Tumor Immunology

In Hodgkin's lymphoma, several molecular defects have been associated with the deregulation of cell proliferation, differentiation, and apoptosis (B. Dörken). Hodgkin-Reed Sternberg (HRS) cells show constitutive activity of transcription factors such as AP-1 and NFκB. The NFκB signaling pathway appears to be partly responsible for the apoptosis resistance of HRS cells. This involves the up-regulation of c-FLIP proteins and the inhibition of the classical cell death pathways involving CD95 and TRAIL. In addition, cell proliferation and apoptosis of HRS cells seem to be influenced by Notch signaling as the interaction between Notch1 on tumor cells and its ligand Jagged1 induces proliferation and inhibition of apoptosis *in vitro.* Remarkably, ligand-induced Notch signaling was also identified as a critical growth factor for cultured and primary multiple myeloma cells suggesting that these interactions contribute to myelomagenesis in the bone marrow microenvironment.

Little is known about how spontaneous, non-viral tumors efficiently manage to escape recognition and elimination by the immune system, a process termed immunosurveillance. The so-called immunoediting hypothesis postulates that tumors escape T cell recognition by losing or down-modulating molecules, which can trigger strong immune responses. In addition, there is evidence that tumors themselves form microenvironments, which paralyze immune cells, like dendritic cells and T cells, necessary for tumor-specific immunosur-

Tumor-Immunologie

In Hodgkin Lymphomen wird die deregulierte Proliferation, Differenzierung und Apoptose von Tumorzellen mit einer Anzahl molekularer Defekte in diesen Zellen assoziiert (B. Dörken). Hodgkin-Reed-Sternberg (HRS) Zellen zeigen beispielsweise eine konstitutive Aktivität der Transkriptionsfaktoren NFκB und AP-1. Insbesondere NFκB kommt dabei eine zentrale Funktion bei der Apoptose-Resistenz der HRS-Zellen zu, die mit einer verstärkten Expression von FLIP Proteinen und der Inhibition klassischer Signalwege der Apoptose über CD95 und TRAIL einhergeht. Zusätzlich scheint die Signalvermittlung über Notch-Rezeptoren das Wachstum von HRS-Zellen zu beeinflussen, da die Interaktion zwischen Notch1 auf der Oberfläche von Tumorzellen und seinem Liganden Jagged1 *in vitro* die Proliferation der Hodgkin-Zellen stimuliert und gleichzeitig die Apoptose inhibiert. Die über Notch vermittelte Signaltransduktion ist darüber hinaus ein wichtiger Faktor für das Wachstum primärer und kultivierter Plasmozytom-Zellen und ist daher vermutlich auch im Mikromilieu des Knochenmarks an der Entstehung von Plasmozytomen beteiligt.

Bisher ist wenig darüber bekannt, wie spontan entstehende, nicht durch Viren verursachte Tumoren der Immunüberwachung entkommen können. Eine Theorie besagt, dass Tumoren wachsen können, indem sie durch „Immunoediting" der Erkennung durch T-Zellen dadurch entgehen, dass sie die Produktion derjenigen Faktoren herunterregulieren oder einstel-

veillance. The development of a novel transgenic animal model, in which the viral cancer-promoting SV40 large T protein is activated randomly in various tissues, allows the analysis of the immune response against sporadic tumors arising from single cells (T. Blankenstein). Interestingly, although these spontaneous tumors retained their immunogenicity after transplantation, they were unable to induce or sustain a protective immune response. Instead, they induced tolerance associated with the expansion of non-functional T cells. Although the underlying mechanisms are yet unknown, these results argue against immunosurveillance of spontaneous cancer.

A critical aspect in cancer immunotherapy is the generation of a strong, tumor-specific immune response. Genetically modified T cells expressing T cell receptors (TCR) specific for tumor-associated antigens may facilitate cancer therapy (W. Uckert). The retroviral vector MP71, based on the mouse myeloproliferative sarcoma virus, yielded up to 75-fold higher transgene expression than a vector based on the Moloney murine leukaemia virus (MLV). This suggests that MP71 is generally applicable for high-expression gene transfer into T cells. This reprogramming of TCR specificity could be used to generate T cells directed against the tumor and thus support cancer therapy.

Autoreactive T cells which have escaped the regulatory mechanisms of the immune system may cause severe autoimmune diseases. Several mechanisms have been described which might control these cells (O. Rötzschke/K. Falk). One of these are regulatory CD25+CD4+ T cells (Treg cells), which constitute a central element of peripheral tolerance. The phenotypic and functional characteristics of these cells are poorly defined with regard to memory. Interestingly, the chemokine receptor CCR6 is expressed on a distinct memory-like subset of mouse and human Treg cells, which are generated in vivo from CCR6-CD25+ T cells after antigen encounter. These CCR6+ Treg cells accumulate in the central nervous system after induction of experimental autoimmune encephalomyelitis (EAE). The positioning and phenotype of these Treg cells suggests that they are meant to control potentially destructive autoimmune responses directly in inflamed tissues.

Homeostatic trafficking of lymphocytes through secondary lymphoid organs as well as extralymphoid tissues is required for immune surveillance and the establishment of self-tolerance (M. Lipp). The homeostatic chemokine receptors CCR7 and CXCR5 control lymphocyte trafficking to and within secondary lymphoid organs. However, their role in regulating lymphocyte recirculation through peripheral tissues under non-inflammatory conditions is not well understood. Remarkably, the chemokine receptor CCR7 also controls homeostatic recirculation of lymphocytes through body cavities and non-lymphoid tissues. Therefore, naïve and memory lymphocytes accumulate in body cavities and mucosal tissues of CCR7-/- mice due to impaired lymphocyte egress via afferent lymphatics (U. E. Höpken). Thus, CCR7 appears to be a key regulator of homeostatic recirculation of lymphocytes through peripheral tissues.

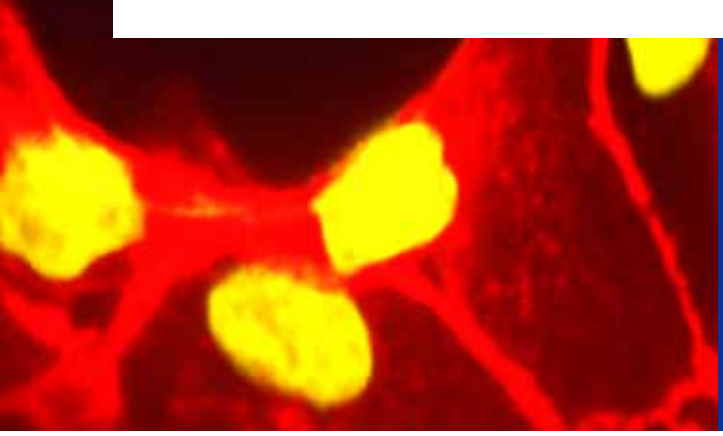
len, die die Immunreaktion anfangs ausgelöst haben. Es gibt aber auch experimentelle Hinweise darauf, dass Tumorzellen selbst ein Mikromilieu schaffen, das Immunzellen, wie dendritsche Zellen oder T-Zellen, in Anergie versetzt und damit lahmlegt. Ein neuartiges transgenes Tiermodell, indem ein virales Onkogen, das sogenannte SV40 T-Antigen, zufällig in unterschiedlichen Geweben aktiviert wird, ermöglicht es, die Immunantwort gegen sporadische, aus einer einzelnen Zelle entstandene Tumore zu untersuchen (T. Blankenstein). Obwohl diese Tumore nach Transplantation in gesunde Tiere noch eine starke Immunantwort hervorrufen können, hatten sie diese Fähigkeit in den ursprünglich erkrankten, transgenen Tieren verloren. Stattdessen entwickelten die betroffenen Tiere eine nicht protektive Immunantwort verbunden mit der Bildung funktionsloser T-Zellen. Diese Ergebnisse zeigen erstmals, dass diese spontanen Tumore nicht durch Immunoediting der Immunüberwachung entkommen, sondern Toleranz hervorrufen.

Ein wichtiger Aspekt der Immuntherapie bei Krebs ist die Generierung einer effizienten Tumor-spezifischen Immunantwort. T-Zellen, die mit Hilfe retroviraler Vektoren genetisch so modifiziert wurden, dass ihre T-Zell-Rezeptoren (TCR) spezifisch ein Tumor-assoziiertes Antigen erkennen, sind für eine adoptive T-Zell Therapie besonders geeignet (W. Uckert). Der von dem myoproliferativen Sarkomvirus der Maus abgeleitete retrovirale Vektor MP71 zeigte eine bis zu 75-fache höhere Expression des Transgens in T-Zellen im Vergleich zu konventionellen Vektoren und konnte damit sehr effizient zur Tumorantigen-spezifischen Umprogrammierung von T-Zellen mit Hilfe rekombinanter TCR eingesetzt werden.

Autoreaktive T-Zellen, die den Kontrollmechanismen des Immunsystems entkommen, können schwere Autoimmunerkrankungen hervorrufen (K. Falk/O. Rötzschke). Ihre Kontrolle wird im Wesentlichen durch sogenannte regulatorische CD25+CD4+ T-Zellen (Treg) sichergestellt, die zwar eine zentrale Bedeutung bei der Aufrechterhaltung der peripheren Toleranz spielen, deren funktionelle Eigenschaften vor allem bezogen auf die Entwicklung eines regulatorischen immunologischen Gedächtnisses aber nur unvollkommen verstanden sind. Eine neue, beim Menschen und der Maus konservierte Subpopulation von Treg-Zellen mit typischen Eigenschaften von Gedächtniszellen ist durch die Expression des Chemokinrezeptors CCR6 charakterisiert und kann aus CCR6-CD25+ T-Zellen nach Antigen-Kontakt generiert werden. Interessanterweise reicherten sich diese CCR6+ Treg-Zellen nach Induktion einer Experimentellen Autoimmun-Enzephalitis (EAE) im Gehirn von Mäusen an und scheinen direkt an der Kontrolle von Autoimmun-Prozessen in entzündeten Geweben beteiligt zu sein.

Die homeostatische Rezirkulation von Lymphozyten durch sekundäre lymphatische Organe aber auch durch nicht-lymphatische Gewebe ist von essentieller Bedeutung für die Immunüberwachung und die Etablierung der sogenannten Selbsttoleranz (M. Lipp). Während die Einwanderung von Lymphozyten und dendritischen Zellen in die T- und B-Zell Zonen sekundärer lymphatischer Organe maßgeblich durch die homeostatischen Chemokinrezeptoren CXCR5 und CCR7 gesteuert wird, weiß man wenig über die Mechanis-

men, die die Migration von Lymphozyten durch periphere Gewebe unter nicht-inflammatorischen Bedingungen steuern. Offensichtlich reguliert CCR7 auch die Migration von Lymphozyten durch nicht-lymphatische Organe. In CCR7-defizienten Mäusen reichern sich deshalb naive und Gedächtnis T-Zellen in der Peritonealhöhle und Mukosa-assoziierten Geweben, wie Magen und Dickdarm, an, da die Lymphozyten nicht über die afferenten lymphatischen Gefäße in die Lymphknoten auswandern können (U. E. Höpken). CCR7 stellt damit ein Schlüsselmolekül für die Regulation der homeostatischen Rezirkulation von Lymphozyten durch peripheres Gewebe dar.



Epithelial Signal Transduction, Invasion, and Metastasis

Walter Birchmeier

The molecular and functional analysis of cell adhesion and signaling pathways in development and tumor progression has been the major focus of our laboratory for many years. We defined vital functions of the E-cadherin/β-catenin system, which is important for cell-cell adhesion of epithelial cells and for preventing invasion and metastasis. Moreover, we discovered that β-catenin, which is also a central component of the canonical Wnt signaling pathway, binds to the transcription factors LEF/TCF. This interaction enables β-catenin to translocate to the nuclei of cells, where it regulates gene expression. We also demonstrated that in the absence of Wnt signals, β-catenin is degraded by a protein complex containing Conductin/Axin, APC, Diversin, GSK3β, and CK1ε. Nuclear signalling of β-catenin is essential in many developmental processes as well as in tumor progression. Mice deficient in β-catenin die early in embryogenesis (at E 8.0) due to defective anterior-posterior axis formation and absence of mesoderm. We have also used conditional gene ablation to study the role of β-catenin in the skin, the limbs, and the brain, and essential functions of β-catenin in these organs have been established. In the skin, β-catenin is essential for specification of stem cells to the hair, but not epidermal lineage. Moreover, our analyses of plakoglobin (γ-catenin) knock-out mice have established a role of this junctional molecule in heart stability and junction formation.

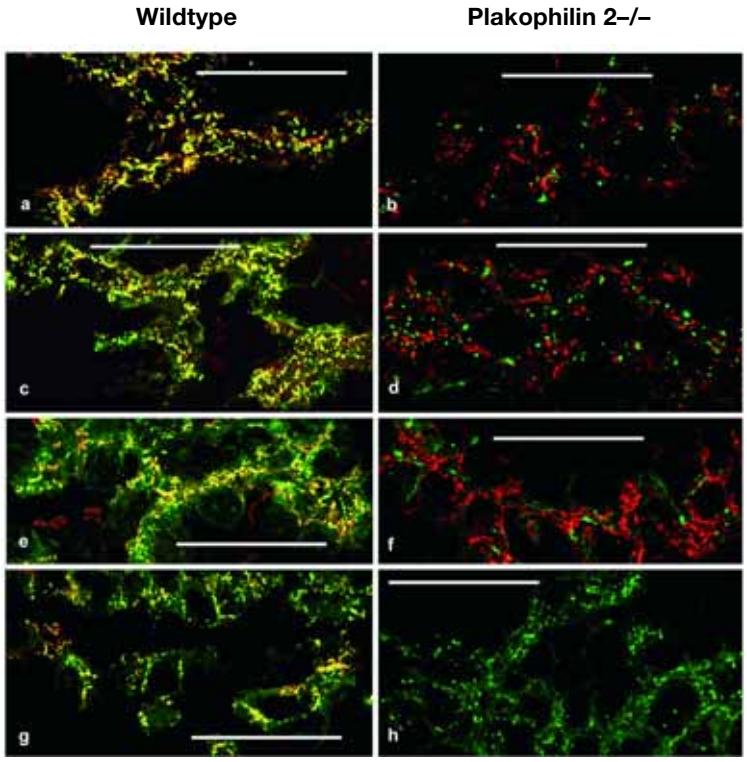
In addition, we have a long-standing interest in the role of scatter factor/hepatocyte growth factor (SF/HGF) and its receptor, the c-Met tyrosine kinase, both of which play a role in tumor progression and metastasis. Signaling elicited by these molecules is vital for the migration and morphogenesis of epithelial cells. An example is the downstream effector Gab1, which mediates the signal responsible for branching morphogenesis of epithelial cells. Gab1-deficient mice die during mid-gestation due to placenta and liver defects as well as show an absence of migration of muscle precursors into limbs.

Requirements of plakophilin 2 for heart morphogenesis and cardiac junction formation

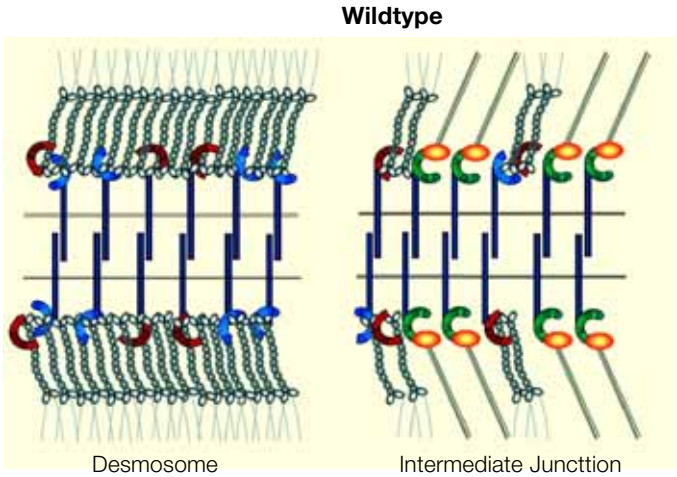
Katja S. Grossmann, Joerg Huelsken, Martin Behrend, Bettina Erdmann, in collaboration with Christine Grund and Werner W. Franke (DKFZ, Heidelberg)

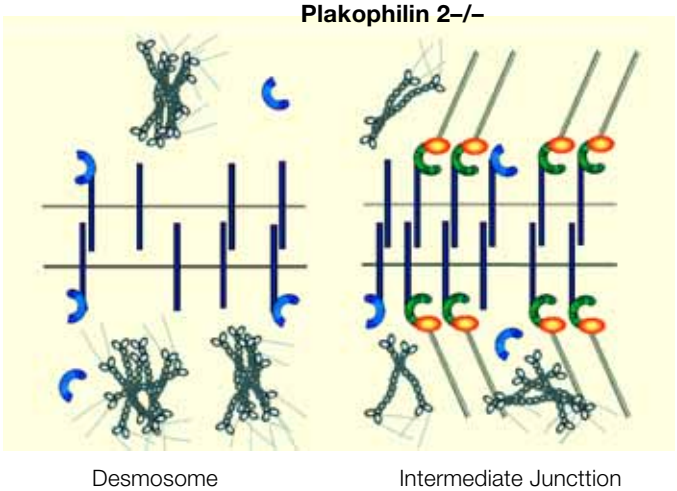
Plakophilins are proteins of the armadillo family (as are β-catenin and plakoglobin) that function in embryonic development and in the adult and, when mutated, can cause disease. We have ablated the plakophilin 2 gene in mice. The resulting mutant mice exhibit lethal alterations in heart morphogenesis and stability at mid-gestation (E10.5–E11), characterized by reduced trabeculation, disarrayed cytoskeleton, ruptures of cardiac walls, and blood leakage into the pericardiac cavity. In the absence of plakophilin 2, the cytoskeletal linker protein desmoplakin dissociates from the plaques of the adhering junctions that connect the cardiomyocytes and forms granular aggregates in the cytoplasm. By contrast, embryonic epithelia show normal junctions. Thus, we conclude that plakophilin 2 is important for the assembly of junctional proteins and represents an essential morphogenic factor and architectural component of the heart.

Hereditary human cardiomyopathies are characterized by impaired myocardial contractility and ventricular dilatation. Mutations in genes coding for components of the contractile apparatus have been identified in cardiomyopathy. After our report that ablation of the plakoglobin gene in mice leads to heart rupture, it was found that Naxos disease is caused by mutation of plakoglobin that results in cardiomyopathy and skin defects. Mutations of desmoplakin and other junctional proteins in humans were recently also found to be associated with complex heart and skin disorders. Based on our studies with mice, it was therefore tempting to speculate that alterations of the plakophilin 2 gene might also impair heart function and play a role in human heart disease. This has now been found by Brenda Gerull, Arnd Heuser, Ludwig Thierfelder, MDC, and other researchers, in collaboration with our laboratory. Arrhythmogenic right ventricular cardiomyopathy (ARVC) is associated with fibrofatty replacement of cardiac myocytes, ventricular tachyarrhythmias, and sudden cardiac death. In 32 of 120 unrelated individuals with ARVC, heterozygous mutations in the plakophilin 2 gene were discovered. In two kindreds with ARVC, disease was incompletely penetrant in most carriers of plakophilin 2 mutations.

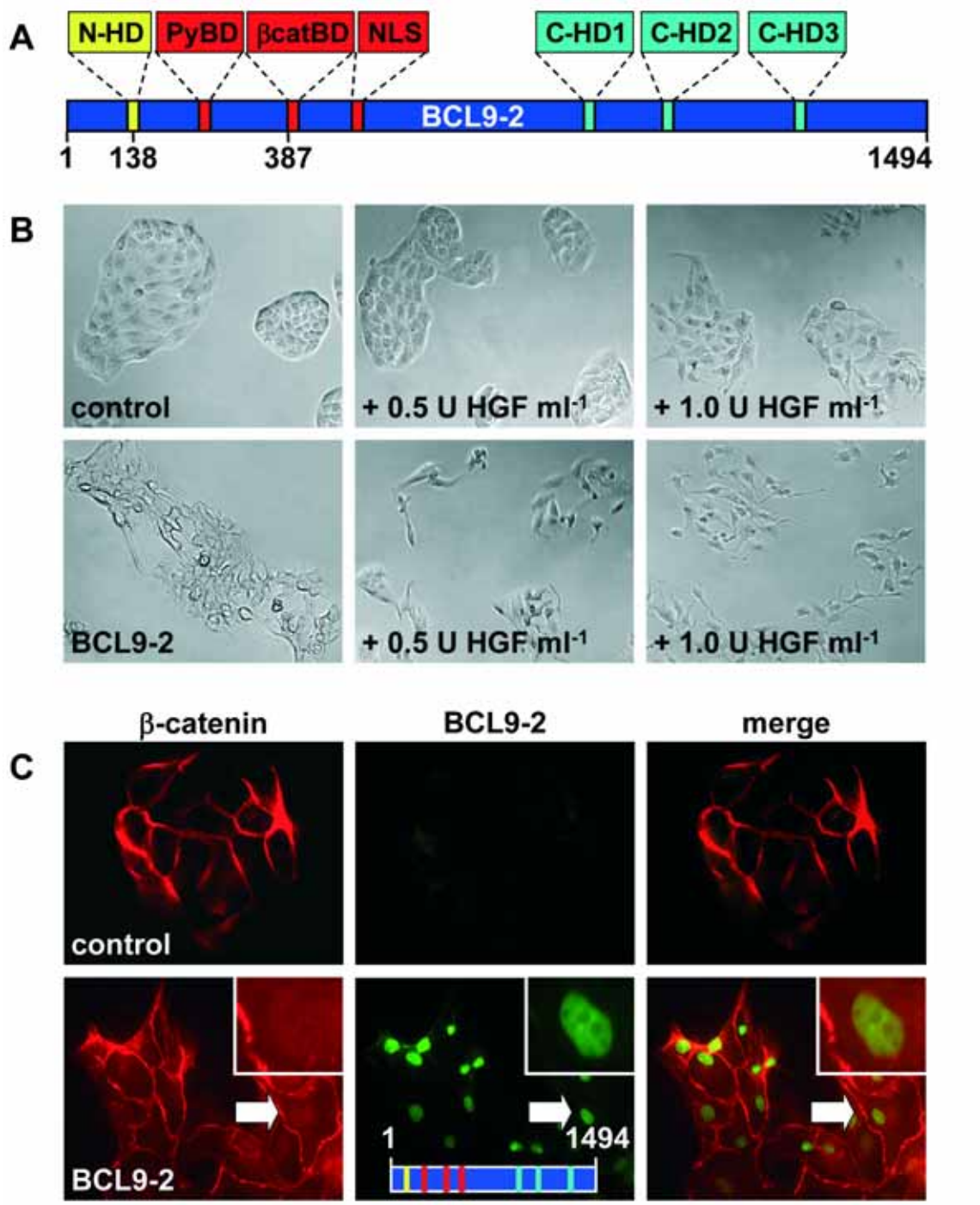


Major changes of the adhering junctions in the intercalated disks of hearts in plakophilin 2-deficient E10.75 mouse embryos.
In immunofluorescence microscopy of cardiac tissue, specific combinations of antibodies were used: desmoplakin (marked by green fluorescence) and antibodies to diverse other cardiac adhering junction components (red fluorescence): **(a, b)** plakoglobin; **(c, d)** N-cadherin; **(e, f)** β-catenin; **(g, h)** plakophilin 2. The merged fluorescence images are shown. In the wildtype embryos, the adhering junctions of the intercalated disks show colocalization (yellow) of desmoplakin with other junctional proteins **(a, c, e, g)**. By contrast, in the plakophilin 2-/- embryos, none of the other plaque proteins colocalizes with desmoplakin **(b, d, f)**. Instead desmoplakin is dispersed over the cytoplasm, often appearing in granular aggregates (green dots). **(h)** Shows the complete absence of plakophilin 2 immunostaining in the mutant embryos. Scale bars, 50 µm.





Schematic structure of cardiac adhering junctions and changes in junctional composition in hearts of plakophilin 2-deficient E10.75 mouse embryos.
In wildtype hearts, typical desmosomes, adherens junctions and intermediate junctions are present. Loss of plakophilin 2 leads to the dissociation of desmoplakin from the junctional plaques. Desmoplakin forms aggregates in the cytoplasm and is often still found to be associated with the intermediatefilament cytoskeleton, that forms unordered swirls around the desmoplakin aggregate. In addition, the expression levels of plakoglobin and desmosomal cadherins are reduced in mutant hearts, so that a stable desmosomal junction is not formed. In contrast typical adherens junction proteins such as the classical cadherin N-cadherin or β-catenin and α-catenin are normally localized to mutant intercalated disks.



BCL9-2 in normal epithelial cells induces epithelial-mesenchymal transition and nuclear translocation of b-catenin. (A) Domain structure of vertebrate BCL9-2 proteins. BCL9-2 contains seven short, highly conserved domains: N-HD, novel N-terminal homology domain; PyBD, pygopus binding domain; β-catBD, β-catenin binding domain; NLS, domain with a classical nuclear localization signal; C-HD1, C-HD2, C-HD3, C-terminal homology domain-1, -2, -3. **(B)** Morphology of normal epithelial MDCK cells that were stably transfected with BCL9-2 or control vector. Cells were also treated with hepatocyte growth factor (0.5 and 1 U ml-1 HGF) for 18 hrs. **(C)** Immunofluorescence microscopy of β-catenin and BCL9-2 in BCL9-2 transfected MDCK cells, and overlapping fluorescence in the nuclei: endogenous β-catenin (red), tagged BCL9-2 (green), and merged fluorescence (yellow).

Essential role of BCL9-2 in the switch between β-catenin's adhesive and transcriptional functions

Felix H. Brembeck, Thomas Schwarz-Romond, Sabine Wilhelm, in collaboration with Jeroen Bakkers and Matthias Hammerschmidt (Max Planck Institute for Immunobiology, Freiburg)

β-Catenin controls both cadherin-mediated cell adhesion and activation of Wnt target genes. We have demonstrated that the β-catenin-binding protein BCL9-2, a homolog of the human proto-oncogene product BCL9, induced epithelial-mesenchymal transitions of nontransformed cells and increases β-catenin-dependent transcription. RNA interference of BCL9-2 in carcinoma cells induces an epithelial phenotype and translocated β-catenin from the nucleus to the cell membrane. The switch between β-catenin's adhesive and transcriptional functions is modulated by phosphorylation of Tyr 142 of β-catenin, which can be induced by SF/HGF and c-Met, favors BCL9-2 binding, and precludes interaction with α-catenin. We have also shown that during zebrafish embryogenesis, BCL9-2 acts in the Wnt8 signaling pathway and regulates mesoderm patterning.

Epithelial-mesenchymal transitions occur during critical phases of embryonic development. Such transitions are also observed late in the progression of carcinomas and provide a possible metastatic mechanism. It was known that epithelial-mesenchymal transitions are initiated by a breakdown of the E-cadherin/β-catenin/α-catenin complex at the plasma membrane and a dissociation of this adhesive complex from the cytoskeleton, which can be induced by tyrosine phosphorylation. We have demonstrated that BCL9-2 contributes to epithelial-mesenchymal transitions and oncogenicity by two mechanisms: (1) interfering with cadherins, which act as tumor suppressor genes, and (2) by increased signaling of family members of the Wnt pathway, which contains many oncogenes and tumor suppressor genes. Furthermore, our data suggest that BCL9-2 acts as a specific modulator of canonical Wnt signaling at particular developmental stages, rather than as a general component of Wnt signaling.

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Genetics of Tumor Progression and Metastasis

Ulrike S. Ziebold
(funded by a
Marie-Curie Excellence Grant)

The central hypothesis our laboratory questions is whether cell-cycle, differentiation and tumorsuppression are innately connected processes. This hypothesis is based on observations that in many tumors uncontrolled proliferation, inappropriate developmental programs, or de-differentiation are observed simultaneously. Since in most, if not all, human tumors the signaling pathway of the retinoblastoma tumor suppressor protein (pRB) is lost or mutated, we believe that this pathway is the critical hinge to explore this tight connection of cell-cycle, differentiation, and tumor suppression. Thus, we are dissecting pRB/E2F3-dependent processes using genetic and molecular tools with an emphasis on the mouse as our model system. First, we rely on genetically modified murine embryonic stem cells (mES-cells) and second, we apply established as well as newly created mouse cancer models.

Using murine embryonic stem cells for a gene-trap screen

Undifferentiated mES-cells have the potential to grow tumors if transplanted into hosts. Once these mES-cells are differentiated, they loose this ability. Concomitant with this change in tumorigenic capacity, mES-cells impose or significantly lengthen their G1/S-phase. This transition of the cell-cycle, central to our hypothesis, is yet poorly defined. Therefore, we have used genechip-micro-array as a first step to monitor all transcriptional changes within the first six days of differentiation, providing companion markers to *oct 3/4* or *nanog*. Currently, we are characterizing this transition phase by devising a gene-trap screen. Next, we want to molecularly dissect the phenotypic changes of this transition using mutant mES-lines of our screen. Lastly, we wish to directly assess *in vivo* functions of promising candidates in the mouse in order to find new molecules and mechanisms that connect the control of embryonic stem cell proliferation, differentiation, and the capacity to form tumors.

The differentiation capacity of tumor suppressors in murine embryonic stem cells

The differentiation capacity of mES-cells is immense. We exploit this capacity for the characterization of G1/S-phase checkpoint molecules using the “hanging drop-method” (pioneered by *A.Wobus*) to form embryoid bodies. These intricate cell-aggregates obtained by differentiation from undifferentiated mES-cells recapitulate many steps of the early mammalian development. With the aid of inducible GFP-tagged transgenes and known differentiation, markers we are characterizing the developmental inducing capacity of tumor suppressors and oncogenes on an individual cell-by-cell base in embryoid bodies.

Finding molecules that regulate progression of tumors and metastasis

In collaboration with P. Schlag RRK/Charité and W. Birchmeier, MDC

We have previously shown that *Rb/E2f3* mutant mice develop aggressive mouse medullary thyroid carcinomas which metastasize to numerous organs. Using these tumors, we hope to gain insight into the nature of common and distinct regulators of metastasis. With micro-array gene-chips, we have compared metastatic and non-metastatic mouse tumors. The differentially expressed transcripts represent novel ‘metastatic markers’. Our candidates are now being tested in functional migration and invasion assays. In the future, we will challenge if these markers are direct pRB/E2F3 target genes and how they impinge upon tumor progression and metastasis.

The tumorigenic capacity of E2F3 in mice and man

In collaboration with R. Bernards, NKI Amsterdam, The Netherlands

We have shown that E2F3, one member of the E2F-family, is a key downstream target of pRB. In addition, we showed that E2F3-loss was able to promote or suppress the development of specific tumors in the mouse. Importantly, over-expression of E2F3 was now identified in a subset of human epithelial tumors (as ovarian cancer). These findings underscore the need to understand the consequences of E2F3 de-regulation. Therefore, we use a knock-in strategy to regulate E2F3 levels in the mouse. This mouse will than be crossed to an ovarian-specific Tamoxifen inducible Cre-line currently being constructed. Ultimately, we wish to realize the full biological tumor functions of E2F3 and pRB.

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Signaling Mechanisms in Embryonic Stem Cells

Daniel Besser
(Helmholtz Fellow)

Embryonic stem cells (ESC) are pluripotent cells which can proliferate indefinitively and participate in the formation of basically all cell types. Recently, researchers have started to analyze human ESCs (hESC) allowing a glimpse into early human embryogenesis and the development of tools for pharmacology and regenerative medicine. While our understanding of the basic biology of mouse ESCs (mESC) is quite advanced, we know very little about the biological properties of hESCs. Studying human cells is of special importance since their properties differ from mouse cells. This finding is not unexpected given the differences in the early embryonic development between mice and humans. One of the foremost questions in the study of hESC is the identification of factors required for maintenance of the pluripotent state. Our laboratory focuses particularly on the maintenance of the undifferentiated, pluripotent state in human embryonic stem cells and the underlying molecular events in the transduction of signals and the regulation of genes. Currently, three major projects are being pursued in the laboratory.

TGFβ and BMP signaling in hESCs

We and others have shown that activation of TGFβ signaling and inhibition of BMP signaling play critical roles in the maintenance of the undifferentiated state in hESCs. We have identified downstream target genes in hESCs for both pathways. TGFβ signaling by the activation of transcription factors Smad2/3 specifically regulates the expression of at least three known target genes (i.e. lefty-A, lefty-B and nodal) while target genes for the BMP pathway via Smad1/5/8 (i.e. id-1 and msx-2) are inhibited in the undifferentiated state and activated immediately upon differentiation. These pathways establish extensive crosstalk between each other and this crosstalk appears to be essential to determine the maintenance of pluripotency and the induction of differentiation.

Moreover, we have shown that a chemical inhibitor of GSK3, 6-bromoindirubin-3'oxime (BIO), which is able to keep hESCs in the undifferentiated state, leads to the activation of TGFβ signaling and inhibition of BMP signaling. Although it has been suggested that BIO by the activation of Wnt signaling pathway maintains the pluripotent state, recent data in our laboratory indicate that the activation of TGFβ signaling and the maintenance of pluripotency appears to be the consequence of a yet unknown target of BIO. The characterization of this new target and its interaction with TGFβ signaling is one major focus of our research and will be important for the understanding of the signaling events in undifferentiated hESCs.

Secreted factors from mouse embryo fibroblasts (MEF)

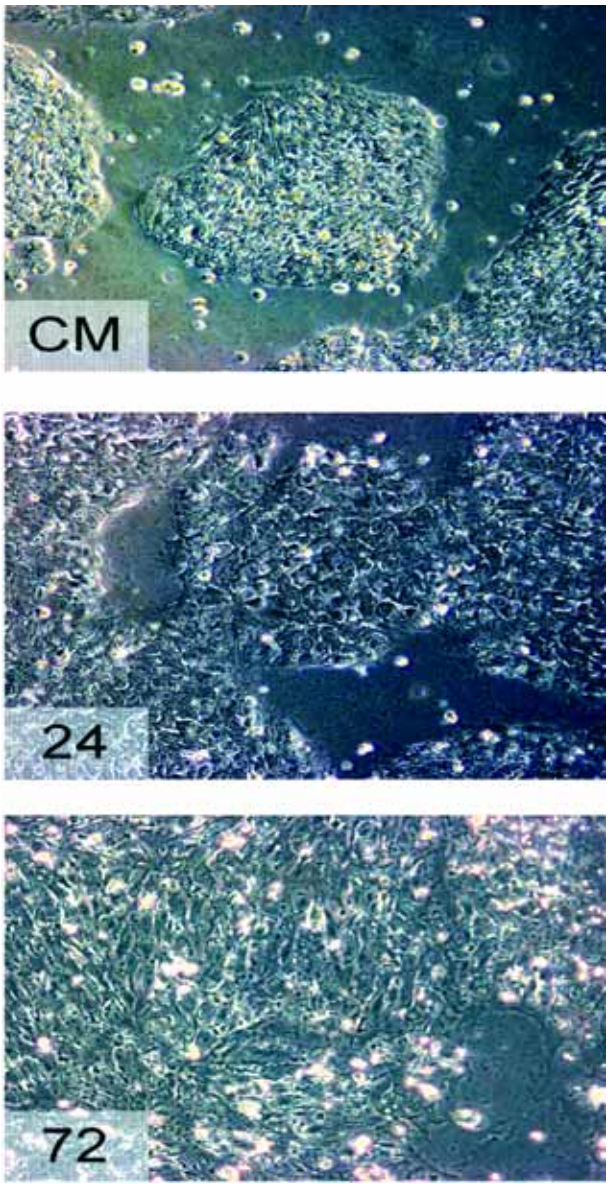
We are also focusing on the identification of unknown secreted factors that are produced by mouse embryonic fibroblasts (MEFs). Conditioned medium from MEFs is sufficient to maintain hESCs undifferentiated suggesting that secreted factors produced by these cells regulate the molecular program in the undifferentiated state of human embryonic stem cells. We found that treatment with the extracellular factor FGF-2 is required to induce the expression of these unknown secreted factors. Using a global expression analysis, we have identified 15 secreted molecules produced by MEFs in the presence but not in the absence of FGF-2 that are candidates for the regulation of the undifferentiated state in hESCs. Expression of these factors will allow us to identify further molecular processes required for pluripotency. We have collected expression plasmids for these factors and are currently expressing them in cell lines which do not support the undifferentiated growth of hESCs. In this project, we also want to establish how these secreted factors influence the regulation of transcription factors which are known to be important for the undifferentiated state of embryonic stem cells (i.e. Pou5F1 (Oct3/4), Sox2, and Nanog).

We are in the process of establishing small hairpin (sh) RNA constructs to allow the knock-down of these factors via expression of small interfering (si) RNAs. This technique will be used to establish a global knock-down approach using shRNA constructs in hESCs and identify further genes that influence the undifferentiated state and the differentiation of the cell into specific cell types. The identification of these factors, signaling pathways, and downstream target genes as well as their crosstalk is of major importance for the future research on hESCs and, ultimately, for their use in pharmacological and medical applications.

Signaling in human mesenchymal stem cells (hMSCs) and their differentiation to cardiomyocytes and hepatocytes

(START-MSC Consortium, BMBF joint project grant)

Recently, we have initiated a project in which signaling mechanisms in hMSCs in their undifferentiated state and during the differentiated into two clinically relevant cell types, cardiomyocytes and hepatocytes, will be studied. The signaling mechanisms in hMSCs, which are potentially of high relevance for cell-based therapies in the near future, will be analyzed as part of a consortium with in vivo approaches, analy-



Differentiation of H1 hESCs on Matrigel. Undifferentiated H1 cells in conditioned medium (CM) and differentiating H1 cells without conditioned medium for 24 and 72 hours. The differentiation can be documented by the flattened morphology of the cells.

ses of structural cell components, genomics and proteomics, and clinical efficacy of the derived cell types. Our findings will also be compared to the molecular events in the undifferentiated state of hESCs, the main focus of the laboratory.

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Yang, E, Lerner, L, Besser, D, Darnell, JE. (2003) Independent and cooperative activation of chromosomal c-fos promoter by STAT3. J. Biol. Chem. 278, 15794-15799

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Identification of genes predictive for human colon cancer metastasis and their involvement in cellular signalling pathways

U. Stein, W. Walther, P.M. Schlag. In cooperation with W. Birchmeier (MDC), E.D. Harris, S.D. Mertins, and R.H. Shoemaker (National Cancer Institute, Frederick, MD), T. Waldman (Georgetown University, Washington, DC), C. Heizmann (University Zürich), D. Allard (University Liverpool, UK)

We focussed on a better understanding of the molecular mechanisms associated with tumor progression and metastasis of colorectal cancer and searched for genes which are involved or causal for these biological processes. We identified a novel gene without significant homology to known sequences in the data bank, referred to as prognostin. It is predictive for colon cancer metastasis formation, with higher expression levels in surgical cancer specimens, which metachronically developed metastases compared to those which did not develop any distant metastasis. The putative protein harbors domains for protein interaction and is involved in the HGF/Met/MAPK-signalling pathway. Prognostin induced cell migration and proliferation in vitro which are reverted by gene-specific siRNA. Enhanced tumor growth and liver metastasis were observed in several in vivo models following transplantation of prognostin-expressing tumor cells.

Other genes such as S100A4/metastasin are known to be highly associated with cancer metastasis. We identified, that S100A4/metastasin is a direct target of the Wnt/β-catenin signalling pathway in colon cancer cells. It induces cell migration and invasion in cell culture, which is knocked down by gene-specific siRNA. S100A4/metastasin has potential value as a predictor of colon cancer metastasis formation in patients. Connection of oncogenic β-catenin signalling with this metastasis-associated gene adds to the existing data on dependent genes related to proliferation or metastasis. Taken together, our findings represent both, identification of genes predictive for metastasis of human colorectal cancer, and identification of targets – genes/signalling cascades they are involved in - for taylor-made intervention strategies with respect to cancer therapy.

On the way to a prognostic chip for colorectal carcinomas

W. Kemmner, U. Stein, J. Fritzmann, P.M. Schlag. In cooperation with M. Morkel, W. Birchmeier (MDC) and Invitek GmbH, Berlin

Colorectal cancer remains a significant health care problem worldwide. To date, the tumor–node–metastasis (TNM) system represents the main tool for identifying prognostic differences. Thus, identifying genes whose differential expression affects the survival of patients after primary tumor surgery is a major aim of clinical cancer research. On the one hand, factors relevant for tumor angiogenesis and glycosylation are examined. To this end, we have developed a oligonucleotide microarray containing probes for 150 of the most relevant glycosyltransferases and carbohydrate metabolizing enzymes. On the other hand, expression profiles of colonic carcinomas have been studied by whole genome microarrays. This analysis led to identification of a number of candidate genes. Evaluation of candidate gene expression by quantitative Taqman RT-PCR supports the results found by GeneChip analysis. Tissue-specific expression of the putative marker genes was also evaluated by in-situ hybridization and immunohistochemistry. Based on data sets from all cooperation projects with the group of Walter Birchmeier, about 300 candidate genes have been selected and spotted for generation of a customized chip. Currently, colorectal carcinomas, available from the comprehensive tumor bank of our hospital, are examined using these low-density chips.

Gene expression profiling of metastasis in colorectal cancer

J. Fritzmann, M. Krause, P.M. Schlag. In cooperation with M. Morkel, W. Birchmeier (MDC) and J. Budczies (GSF Munich)

Much has been learned in recent years about mutations that control the initiation and progression of colorectal cancer but less is known about molecular events that are crucial in metastasis formation. As metastasis is the main cause of mortality in individuals with cancer, the identification of genes that are important for this process is essential. Gene expression profiling of 91 human, locally advanced colorectal adenocarcinomas has been used to identify genes whose expression characterizes metastatic tumors. Therefore RNA was isolated from laser captured microdissected tissue and examined using Affymetrix expression profiling. We found consistent differences in the expression profiles of metastatic primary tumors and lymphatic, hepatic, and pulmonary metastases when compared to non-metastatic tumors. Our findings suggest the existence of a common gene expression signature that indicates the metastatic potential of a large subset of colorectal tumors and metastases. Using a classifier based on this signature, we were able to distinguish metastatic and non-metastatic cancer with high accuracy. Using in situ hybridisation, we confirmed the differential expression of several metastasis-associated genes. This metastasis classifier may therefore provide a strategy to select patients with a high risk of metastasis and thus

appropriate candidates for chemotherapy after surgical resection of the primary tumor.

In vivo application of heat-induced nonviral gene therapy for tumor treatment

W. Walther, U. Stein, P. M. Schlag. In cooperation with EMS Medical Systems SA, Nyon, Switzerland and EPO GmbH, Berlin

We have characterized in detail the proximal human multidrug resistance gene 1 (MDR1) promoter and identified heat-shock (HSE) elements. In vitro studies showed, that these elements permit heat-inducibility by specific binding of heat shock factor-1. This particular feature can be exploited for the construction of heat-regulable expression vectors. We therefore established vector constructs, in which the HSE-harboring portion of the mdr1-promoter is driving the expression of the human tumor necrosis factor alpha (TNF) gene. For the intratumoral in vivo gene transfer, the nonviral jet-injection technology was employed to efficiently transduce naked vector-DNA. *In vivo* experiments, in which colon carcinoma bearing mice were jet-injected with the heat-inducible vectors, revealed the significant heat-induced, temperature-, and time-dependent increase of TNF-expression at mRNA- and protein level. More importantly, the therapeutic approach of combined hyperthermia-induced TNF-expression and adriamycin treatment demonstrated significantly reduced tumor growth associated with tumor necrosis of large tumor portions. In contrast, animals which were treated with either hyperthermia or adriamycin, showed only mild reduction in tumor growth. Therefore, gene therapy of heat-induced TNF-expression sensitizes colon carcinomas towards drug treatment resulting in an improved therapeutic efficacy. This supports the concept that heat-induced MDR1-promoter-driven expression of therapeutic genes is feasible for combined cancer gene therapy approaches.

Mechanisms of lymphatic metastasis of solid tumors with special focus on lysophospholipid- and chemokine receptors

T. Schulze, P.M. Schlag. In cooperation with M. Lipp (MDC)

Lymphatic metastasis at diagnosis is present in up to 25% of patients suffering from gastric cancer. The metastatic spread is in the majority of cases responsible for the fatal outcome of the disease. The molecular mechanisms leading to metastasis appear to occur early in tumor development. We used laser capture microdissection to obtain highly purified cell populations from node-negative and node-positive primary gastric tumors as well as from the corresponding normal gastric mucosa and subsequently performed gene expression profiling to identify candidate genes implicated in the development of lymph node metastasis. Non-supervised clustering of expression profiles from tumor tissue revealed clearly distinguishable profiles from tumors with and without lymphatic metastasis. In contrast, expression profiles from normal gastric mucosa of patients with and without lymph node metastases could not be differentiated. By comparison of expression profiles from metastatic and non-metastatic primary tumors, we were able to define a significant number of genes at least 3 fold up- or downregulated in metastatic gastric cancer. The detailed biostatistical analysis is currently ongoing.

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Molecular Genetics of Cell Differentiation & Tumorigenesis

Achim Leutz

Introduction

Stem cells in the bone marrow continuously replenish huge amounts of exhausted hematopoietic cells including erythrocytes, granulocytes, macrophages, and other specialized blood cell types. Stem cells can also sustain their own maintenance - a condition called self-renewal. Defective control of self-renewal or dysregulated cell differentiation in the hematopoietic system may cause diseases such as leukemia, anemia, or immune defects. Unraveling the conditions and requirements for maintaining stem cells *in vitro*, directing their self-renewal and differentiation are ultimate goals in biology, regenerative medicine, and oncology.

In experimental hematology, cells of different blood cell lineages and maturation stages can be distinguished by specific antibodies that bind to the cell surface. Moreover, differentiation of hematopoietic cells from progenitors can be recapitulated *in vitro*. Hematopoietic stem cells of the mouse can be transplanted into different animals which also permits the engraftment into genetically different hosts. Thus, experimental hematopoiesis provides valuable tools and unique experimental opportunities to address biologically and clinically relevant and fundamental questions related to stem cell biology, lineage commitment, progenitor proliferation, and cell differentiation control.

Combinatorial control of gene expression

Cell differentiation programs are selected by switching on and off distinct sets of genes. Gene regulatory proteins (transcription factors) are downstream of signaling cascades and bind to control regions of developmentally important genes to suppress or activate their expression. Specificity is primarily achieved by combinatorial interactions between several different transcription factors which are simultaneously required for the regulation of the same target genes. Combinatorial gene switches permit plasticity of regulation and multiple developmental decisions to be accomplished with a limited number of regulators. Many such important gene regulatory proteins, however, are also prone to tumorigenic conversion by mutagenesis.

Several years ago, we were able to identify the first combinatorial hematopoietic switch that instructs cells to express granulocytic and macrophage genes. The switch consists of two types of transcription factors that are also involved in leukemogenesis: CCAAT/Enhancer Binding Proteins (C/EBPs) and the product of the c-Myb proto-oncogene. C/EBPs regulate cell differentiation and cell cycle arrest, whereas Myb protein is essential for precursor cell expansion and maintenance of all hematopoietic lineages. In a concerted action, both transcription factors balance proliferation versus differentiation and instruct myeloid gene expression. Mutations in either transcription factor may abrogate their collaboration, disrupt their ability to induce cell differentiation, and contribute to leukemia. This concept has meanwhile been extended to several other co-operating hematopoietic transcription factors in different cell lineages.

Chromatin remodeling and lineage specific gene expression

The machinery involved in gene regulation has to deal with the repressive effects of chromatin. The basic units of chromatin are octameric histone particles, termed nucleosomes, that serve to spool up and package DNA into a transcriptionally inactive form. The last 10 years of research in chromatin and gene regulation has uncovered a plethora of enzymes and molecular protein machinery which alter the structure of chromatin by covalent histone modifications and DNA methylation in order to activate genes or to shut them down. Moreover, some of the chromatin modifications are thought of as manifesting the epigenetic history of individual cell development.

A prerequisite for gene activation is overcoming the repressive effects of chromatin, which requires extensive chromatin remodeling at those genes. C/EBPα and -β were found to interact with the chromatin remodeling SWI/SNF complex. The SWI/SNF complex uses ATP to alter the structure of nucleosomes and to wind DNA around it. The interaction between SWI/SNF and C/EBP is required to modify chromatin in such a way that silent differentiation genes can be activated. Moreover, interaction with SWI/SNF is also required for C/EBP mediated proliferation arrest. As C/EBPs participate in many cell specification events, recruitment of SWI/SNF may represent a major determinant of cell lineage commitment and terminal differentiation.

Mediator: A connection between Ras signaling and C/EBP, activation

C/EBPβ is an intrinsically inhibited transcription factor that acts as a repressor in the absence of signaling which is then turned into an activator by the activated Ras oncoprotein. C/EBPβ is phosphorylated through the Ras/MAP-kinase pathway and this phosphorylation event is accompanied by a conformational change of C/EBPβ. We have found that active

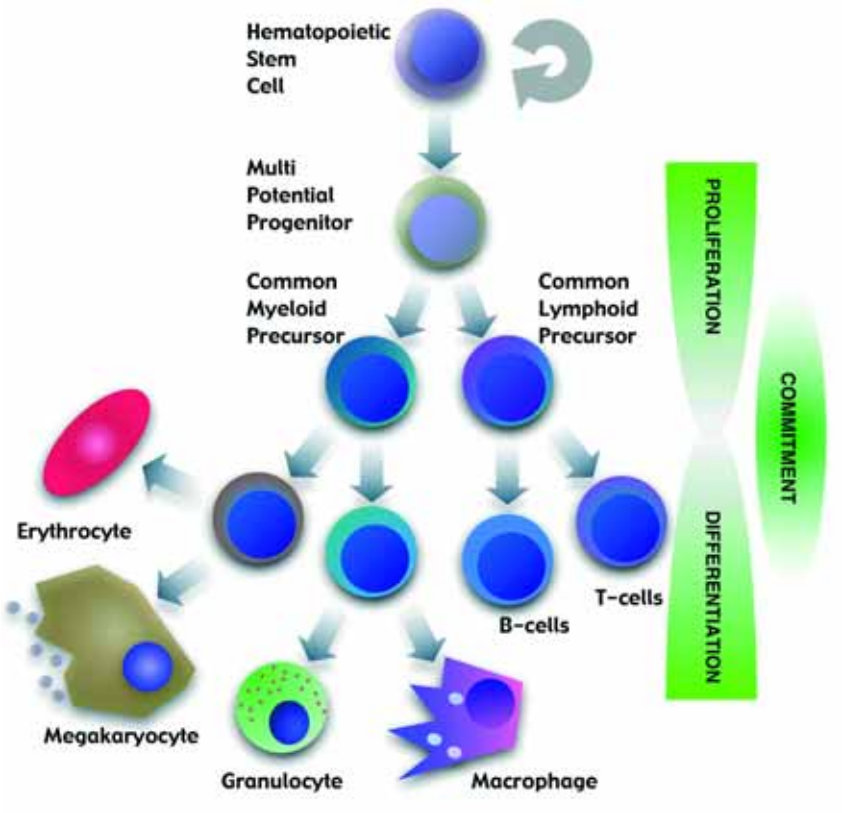

Figure 1. Schematic representation of hematopoiesis
Hematopoietic stem cells in the bone marrow can either self-renew (grey arrow on the right) or give rise to progenitor cells that generate precursors of the myeloid or the lymphoid lineage. The commitment process is characterized by massive cell proliferation in the early phase followed by successive restriction to distinct cell lineages and to cell differentiation. These processes are regulated by trans-acting factors which activate or repress genes and lay down epigenetic changes in the chromatin of cells. This way, cells remember what they are, where they came from, and where they need to go. Leukemic mutations interfere with transcription factor functions, abrogate cell differentiation, and support proliferation. As a consequence, the blood is flooded with immature, non-functional cell types.

and repressive C/EBPβ interacts with two different types of evolutionary conserved multi-subunit complexes that have been termed "Mediator" and that connect transcription factors with the basic transcription machinery, including polymerase II. C/EBPβ preferentially binds to repressive Mediator in its repressive form, whereas Ras signaling selects the transcriptionally active Mediator complex which also associates with RNA polymerase II. This suggests that a Ras-induced structural alteration of C/EBPβ determines differential gene activation through selective interaction with distinct Mediator complexes.

A chromatin function of c-Myb

C/EBPs act in concert with other transcription factors, such as with the Myeloblastosis protein c-Myb in the hematopoietic system or with PPARγ in adipogenesis. The c-Myb transcription factor coordinates proliferation and differentiation of hematopoietic precursor cells. Myb has three consecutive N-terminal SANT-type repeat domains, two of which form the DNA binding domain. Distinct amino acid substitutions in the DNA binding domain alter the way Myb regulates genes and determine the leukemogenicity of a retrovirally transduced v-Myb oncogene, already discovered 65 years ago. How the mutations of the v-Myb oncoprotein unleash its leukemogenic potential was unknown in the past. We have now discovered that the DNA binding domain of Myb also interacts with the N-terminal tails of histones H3 and H3.3. C-Myb facilitates histone tail acetylation by the p300 acetyl transferase which is mandatory during activation of genes involved in differentiation. Albeit the leukemogenic version of Myb binds to the same consensus sequences of target genes, it inhibits cell differentiation, is defective for H3 tail binding and also fails to assist in histone acetylation. Pharmacologic enhancement of H3 acetylation by blocking histone deacetylase activity with Trichostatin A in v-Myb transformed cells restored the activation of maturation genes and induced cell differentiation, implying an epigenetic function of Myb in normal hematopoiesis and in leukemogenesis.

Translational regulation of transcription factors

Different protein isoforms with truncated N-termini may arise by initiation of translation at alternative start sites from several hematopoietic transcription factor mRNAs. The resulting transcription factor isoforms harbor unique N-terminal

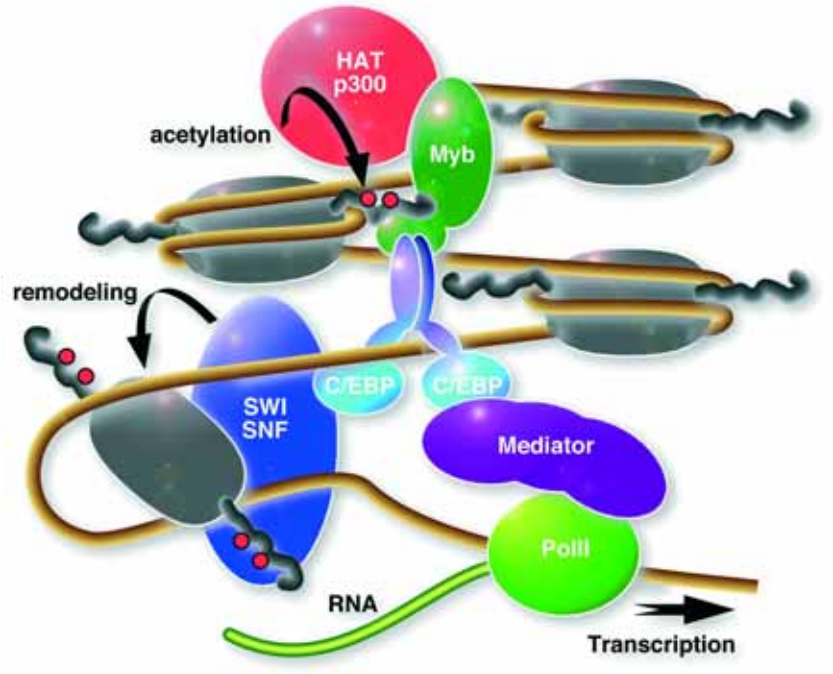

Figure 2. Scheme of Factor Interactions at Myeloid Genes.
Nucleosomal array (nucleosomes: grey spheres; histone H3 tails protrude from nucleosomes; DNA: golden thread) with Myb and C/EBP bound to their cis-regulatory target sites on DNA. Myb and C/EBP interact through the Leucine Zipper (C/EBP) and the DNA binding SANT domain of Myb, respectively. Myb also interacts with H3 N-terminal tails and positions them for acetylation (Histone Acetyl Transferase Complex, HAT, including p300; red sphere). SWI/SNF associates with distinct C/EBP forms and remodels nucleosomes and DNA (lower left). H3 acetylation and SWI/SNF remodeling are both required for subsequent gene activation catalyzed by the interaction with Mediator complex (magenta sphere) that bridges between transcription factors and polymerase II (pol II: green sphere, synthesis of RNA: green strand)

domains which may bind different co-factor complexes with distinct functions in gene regulation and chromatin remodeling. Hence, regulation of alternative translation initiation may play a crucial role in the control of cell fate. We have shown that this is the case with C/EBPα, -β and the stem cell and T-cell leukemia transcription factor Scl/Tal1. Distinct isoforms of these two transcription factors were found to display specific functions in proliferation control, in the activation of genes, and in cell differentiation.

Hodgkin lymphoma and anaplastic large cell lymphoma cells were found to express large amounts of the truncated C/EBPβ isoform, LIP (in collaboration with B. Dörken/F. Jundt). The immunosuppressive macrolide antibiotic rapamycin inhibits the mTOR signaling pathway. It was found that rapamycin has a profound anti-proliferative effect in Hodgkin lymphoma and in anaplastic large cell lymphoma cells. Moreover, rapamycin was shown to down-regulate expression of alternatively initiated truncated isoform of C/EBPβ known to disrupt terminal differentiation and to induce a transformed phenotype. Ectopic expression of the truncated C/EBPβ isoform indeed abrogated rapamycin-induced proliferation arrest, suggesting an involvement of translational regulation of C/EBPβ in both types of leukemia and the pharmacological inhibition of the mTOR pathway as a novel treatment option.

It is anticipated that pathways and factors involved in the control of translational initiation may play far more important roles than previously recognized and that they also represent novel targets for innovative drug therapies. Accordingly, a screening system for drug discovery was developed. Moreover, we have generated genetically altered mouse strains which omit regulatory elements of alternative initiation in order to study the physiological consequences of translational dysregulation of pivotal transcription factors.

Transcription co-factors

Transcription factors interact with other trans-regulatory proteins which function as co-activators, co-repressors, chromatin remodeling factors, and/or bridging factors between gene regulatory complexes. The latter proteins are the “missing links” in the chain of biochemical cascade during chromatin remodeling and in understanding the complexity of gene regulation. We are therefore searching for proteins that interact with hematopoietic transcription factors and oncoproteins. We have been using protein purification affinity protocols and arrayed protein expression libraries on a proteomic scale towards this goal, and were thus able to identify a number of interesting proteins, e.g., proteins that harbor domains implicated in chromatin regulation. We are currently developing murine knock-outs and knock-ins as well as RNAi strategies to determine the effects of C/EBP mutants defective for interactions with several of these co-factors.

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Cancer Stem Cells and Transcription Factors

Frank Rosenbauer

The cancer stem cell concept

Analogous to the development of tissues from normal stem cells, there is increasing evidence suggesting that malignancies are sustained by cancer stem cells, a minor tumor subpopulation which maintains the uncontrolled production of less malignant neoplastic daughter cells (blasts). Cancer stem cells appear to share important functions with normal stem cells such as self-renewal, differentiation, and long-term survival. It is therefore believed that a similar set of critical genes controls both normal and tumor stem cells, which include the Wnt-pathway and the Polycomb-family member Bmi1. The existence of cancer stem cells is of great clinical relevance since their unique "stemness" properties are likely enabling them to escape conventional anti-cancer therapy designed to target the fast cycling and highly proliferating cancer blasts. This inability to eradicate cancer stem cells might be responsible for the disease relapses frequently observed in cancer patients.

Transcription factors orchestrate hematopoietic development

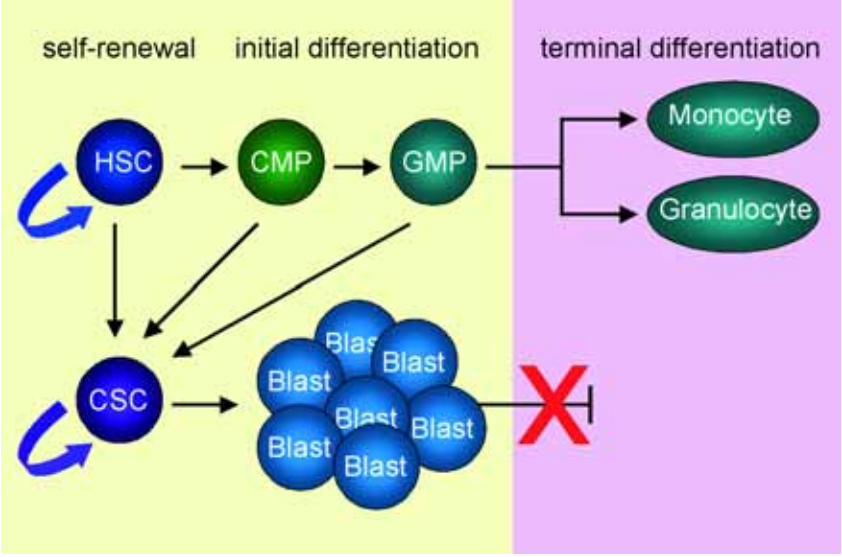
The hematopoietic system is an ideal model in which to study tissue development from normal stem cells and the formation of neoplasms (leukemias and lymphomas) from cancer stem cells. Differentiation of hematopoietic stem cells (HSCs) and progenitors is under strict control of a regulatory network orchestrated by lineage-specific transcription factors. Among the best-studied examples are PU.1, CCAAT/enhancer-binding protein α (C/EBPα), AML-1, GATA-1, c-myb, and SCL/Tal-1. Mice in which these genes have been knocked out display profound hematopoietic defects. Moreover, these transcription factors have been 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 toward 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.

PU.1 is a central regulator of all hematopoietic lineages and stem cell activity

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. Our current research is focused 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, since enforced PU.1 expression in thymic organ cultures completely blocked T cell production. 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 key to deciphering its role in normal hematopoiesis and malignant transformation.

Dynamic PU.1 expression pattern is controlled by a key regulatory DNA element

We reported previously that the proximal PU.1 gene promoter is insufficient for reporter gene expression in transgenic mice, indicating that additional elements are required for PU.1 gene regulation *in vivo*. In support of this, three clusters of DNaseI hypersensitive sites harboring potential regulatory elements were identified in the PU.1 gene locus. Inclusion of the most distal cluster, located 14 kilobases upstream of the PU.1 gene transcription start site (referred to here as URE for upstream regulatory element), with the proximal PU.1 promoter resulted in reporter gene expression in transgenic animals in the same cells that express endogenous PU.1. To further analyze the role of the URE in regulation of the endogenous PU.1 gene *in vivo*, we generated URE deficient mice ($URE^{\Delta/\Delta}$) using targeted recombination in ES cells. Remarkably, URE deletion led to a 5-fold 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 inter-



Stem cell functions are shared by normal hematopoietic stem cells and cancer stem cells: As demonstrated by the pathogenesis of acute myeloid leukemia (AML), both normal hematopoietic stem cells (HSC) and neoplastic cancer stem cells (CSC) have the ability to self-renew and differentiate into less pluripotent daughter cells. However, while HSCs produce short-lived progenitors, such as common myeloid progenitors (CMP) and granulocyte monocyte progenitors (GMP), which terminally differentiate into mature monocytes and granulocytes, CSCs in AML give rise to leukemic blasts, which harbor a block in their terminal differentiation. Recent experiments using murine transplant models suggest that both HSCs and committed myeloid progenitors can transform into a CSC.

ference 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.

We have identified several highly conserved binding motifs for transcription factors in the URE and are currently engaged in investigating which of these factors are essential for regulation of URE activity, thus directing the cell type specific expression pattern of PU.1. Revealing the PU.1 upstream pathways is pivotal in understanding how a single transcription factor can orchestrate the development of multiple lineages and can initiate cancer. Furthermore, we have begun experiments to prospectively isolate and characterize the cancer stem cell compartment in diseased $URE^{\Delta/\Delta}$ mice. These studies will contribute significantly toward our understanding of the unique molecular properties of these highly malignant cells and may allow us to develop more selective and targeted anti-cancer therapies in the future.

Selected Publications

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Rosenbauer, F, Koschmieder, S, Steidl, U, and Tenen, DG. (2005). Effect of transcription factor concentrations on leukemic stem cells. Blood. In press.

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Signal Transduction in Tumor Cells

Claus Scheidereit

Our laboratory is interested in understanding how the diverse cellular signal transduction processes result in ordered changes of gene expression. Transcription factor "nuclear factor kappaB" (NF-κB) is a model system with wide physiological and medical relevance for humans. A major effort is to decipher the mechanisms and structures that determine gene regulation by NF-κB, its crosstalk with other gene regulatory systems, and to dissect both its role in development and in the pathogenesis of diseases.

Molecules and mechanisms that control NF-κB activity

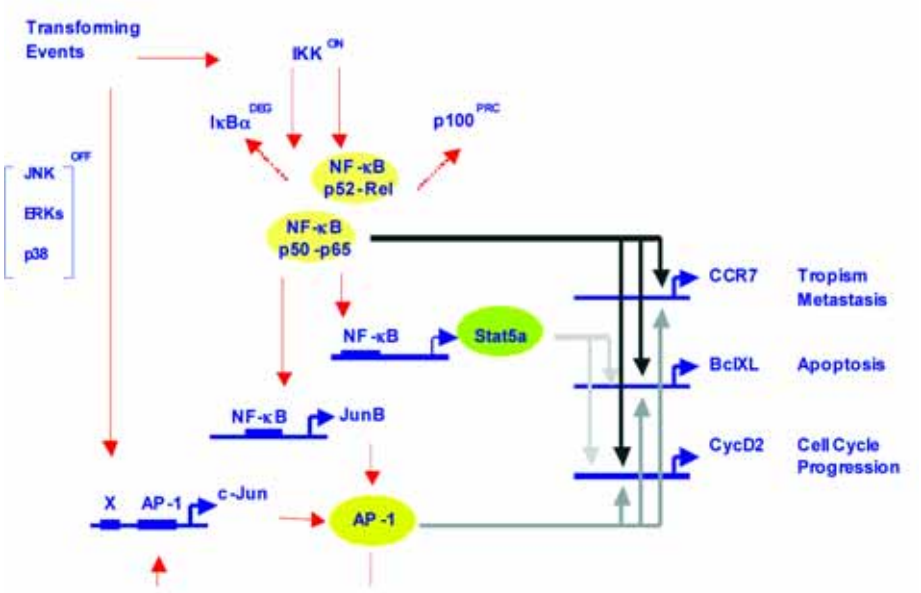
As a pleiotropic gene regulator, NF-κB is present in most if not all cell types of the body and regulates the expression of numerous genes. These encode cytokines, surface receptors, adhesion molecules, transcription factors, and other functional classes of proteins. Biological processes which involve NF-κB activation include the innate and adaptive immune responses, inflammation, and 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, including microbial pathogens, cytokines, mitogens, 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 proteolytic destruction and liberation of active NF-κB.

Mammalian NF-κB comprises five related members, p50, p52, p65, c-Rel, and RelB. They form distinct hetero- and homodimers and bind to inhibitory cytoplasmic IκB molecules, IκBα, β or ε, or to the nuclear IκB homologues Bcl-3 and MAIL. As a characteristic feature of NF-κB, 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.

In stimulated cells, the fate of the NF-κB precursors is controlled by IKK complexes. On stimulation with pro-inflammatory agents, such as tumor necrosis factor (TNFα) or bacterial lipopolysaccharides (LPS), cellular p105 is phosphorylated by IKKs at its C-terminus, resulting in association of βTrCP type SCF ubiquitin ligases. Subsequent to the resulting polyubiquitination, p105 is completely degraded by the proteasome and releases associated NF-κB subunits. As such, p50, which is continuously formed by basal p105 processing, is then liberated to migrate to the nucleus and affect gene expression. In contrast to the signal-induced complete degradation of p105, the structurally similar p100 molecule undergoes stimulus-dependent processing. Certain inducers, including lymphotoxin and LPS, but not other activators of classical NF-κB, such as TNFα or IL-1, trigger proteolytic maturation of cytoplasmic p100 to its p52 product. This processing reaction involves IKK-induced p100 polyubiquitination and partial p100 digestion by the proteasome and takes place while or shortly after p100 is synthesized by the ribosome. The exact mechanisms underlying p100 maturation are under further investigation.

IKK/NF-κB signaling and constitutive AP-1 and Stat5 transcription factor activities in Hodgkin lymphoma pathogenesis

In collaboration with the research department of Bernd Dörken, we are investigating the origins and the biological functions of constitutively activated NF-κB and its downstream effector networks in Hodgkin lymphoma (HL) tumor cells and in related T or B cell malignancies. The NF-κB system is dysregulated in a cell-autonomous manner, generally involving a persistent activation of the IKK complex, which results in constitutive release of NF-κB complexes via both canonical and p100 pathways. Furthermore, Bcl-3 expression is highly activated. Using microarray analyses, we have determined the NF-κB target gene signature in HL cells and could show that constitutive NF-κB drives expression of genes which regulate cell cycle progression inhibit programmed cell death, direct tropism and migration of tumor cells, and their resistance to chemotherapeutic drugs and abundant cytokine production. Thus, a central pathogenic role of the IKK/NF-κB pathway is likely. In addition to IKK/NF-κB, Hodgkin's lymphoma cells reveal aberrant activation of transcription factor AP-1 (activating protein 1) composed of the c-Jun and JunB subunits. While JunB is upregulated by NF-κB, c-Jun is regulated by an independent mechanism. AP-1 cooperates with NF-κB to superactivate a subset of NF-κB target genes in HL. Furthermore, Stat5 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 LPS-stimulated pre-B cells, we could show that LPS-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's lymphoma.



Hypothetic scheme of signaling pathway integration of constitutive IKK/NF-κB and AP-1 with subordinate transcription factors (STAT5a) in Hodgkin lymphoma cells. One or more initial stimuli activate IKK and c-Jun. The latter is independent of MAP kinases (ERK, p38, JNK). Activated NF-κB determines the composition of AP-1 (JunB expression), as well as expression and activation of STAT5a. The network acts on common target genes, whose products affect dissemination, proliferation, and apoptosis of the tumor cells.

Role of the chaperones Hsp90 and Cdc37 for inducible and constitutive IKK and NF-κB activation

The IKK complex is associated with Hsp90 and Cdc37. We observed that the ATPase function of Hsp90 is required for transient IKK kinase and NF-κB activation by a variety of agents and for constitutive IKK activation in Hodgkin lymphoma cells. Hsp90 ATPase inhibition rapidly attenuates the enzymatic activity of IKK and, with slower kinetics, interferes with IKKα and IKKβ *de novo* synthesis. Hsp90 function is required to prevent ubiquitination and proteasomal degradation, presumably during co-translational folding. We could show that pharmacological Hsp90 inhibition enhances programmed cell death of HL cells in an IKK-dependent manner. Further studies aim to dissect the mechanism by which Hsp90 and Cdc37 control IKK activation.

Genetic models to study the function of NF-κB in epithelial organogenesis and in cardiovascular diseases *in vivo*

With a conditional gene targeting approach, we have previously designed a mouse model ($c^{IκBαΔN}$) that allows the down-modulation of NF-κB activity in the entire organism or in restricted cell types and organs. Using this system along with transgenes carrying an NF-κB-dependent *β*-galactosidase reporter gene, we could show that NF-κB is required for the early embryonic development of hair follicles, eccrine glands, and molar teeth and for the formation of secondary lymphoid organs like Peyer's patches and peripheral lymph nodes. The epidermal phenotype of mice with suppressed NF-κB reflects the same defects as observed in the inherited disease "hypohidrotic ectodermal dysplasia" in humans. In ongoing studies, we are investigating the integration of NF-κB into morphogenic networks in the epidermis, including the pathway formed by the ligand ecodysplasin (EDA) and its receptor (EDAR).

The $c^{IκBαΔN}$ system has also enabled us to investigate the in vivo role of NF-κB in the pathogenesis of cardiovascular diseases. In collaboration with the group of Martin W. Bergmann, we have generated mice with a heart-restricted NF-κB down-modulation. When challenged with angiotensin II or the beta-adrenoceptor agonist isoproterenol to induce hypertension and subsequent cardiac hypertrophy, cardiac NF-κB inhibition was sufficient to abrogate heart muscle hypertrophy. This protective effect of NF-κB suppression was attained without detectable damaging effects, such as increased apoptosis of cardiac muscle cells.

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charide-induced gene expression and control subordinate activation of AP-1. Mol. Cell. Biol. 24, 6488-6500.

Freund, C., Schmidt-Ullrich, R., Schneider, W., El-Jamali, A., Löser, P., Dietz, R., Scheidereit, C., and Bergmann, M.W. (2005) Requirement of NF-κB in angiotensin II - and isoproterenol - induced cardiac hypertrophy in vivo, Circulation 111, 2319-2325.

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Intracellular proteolysis

Thomas Sommer

The secretory pathway of eukaryotic cells harbors an elaborate protein quality control system which prevents the accumulation of misfolded or unassembled proteins in the secretory pathway. This system is localized in the Endoplasmic Reticulum (ER). ER associated protein degradation (ERAD) is an important component of this quality assurance system and directs misfolded proteins for destruction by the cytoplasmic ubiquitin-proteasome pathway (UPS).

ERAD can be divided mechanistically into separate steps: First, misfolded proteins are detected within the ER-lumen, a step that, most likely, requires molecular chaperones. Second, the proteolytic substrates are targeted to and inserted into an aqueous transport channel that probably includes the multispanning membrane protein Sec61p. Third, the substrates are transported back into the cytosol in a process termed dislocation or retro-translocation. Fourth, a polyubiquitin chain is synthesized on the dislocated substrates. This step requires the action of membrane-bound components of the ubiquitin system. In yeast, these are the ubiquitin-conjugating enzymes Ubc1p, Ubc6p, and Cue1p assembled Ubc7p and the ubiquitin ligases Hrd1p and Doa10p. Fifth, the Cdc48p/Ufd1p/Npl4p ATPase complex helps to release the ubiquitin-conjugated substrates from the ER-membrane. Finally, the cytosolic 26S-proteasome digests the ubiquitin-conjugated dislocated molecules.

Our group has defined the components and the basic principles of ERAD in the last decade. This process seems to occur in all eukaryotic organisms in a highly conserved manner. Malfunctions in this system lead to altered protein composition in the secretory pathway and may therefore cause serious human diseases like Cystic Fibrosis and Parkinson's disease. Moreover, it has been suggested that some human viruses co-opt the ERAD systems to destroy specific host proteins and manifest themselves in the infected cell.

Recently, we have identified a new component necessary for proteolysis of yeast ER-proteins. This factor, Ubx2/Sel1p, recruits the Cdc48p-complex to the ER-membrane-bound ubiquitin ligases Doa10p and Hrd1p. In the absence of Ubx2/Sel1p, both the association of the Cdc48p-complex and the interaction of Doa10p with the cognate ubiquitin-conjugating enzymes remain intact. Thus, Ubx2/Sel1p mediates the interaction of the assembled Cdc48p-complex with the ERAD ubiquitin ligases Doa10p and Hrd1p. Proteolysis of membrane-bound and luminal ERAD substrates is strongly reduced in cells lacking Ubx2/Sel1p, demonstrating that the Ubx2/Sel1p mediated interaction of the Cdc48p/p97 complex with the ubiquitin ligases is crucial for ERAD. This view is further strengthened by the fact that in ubx2/sel1 knockout cells the ER-stress response (unfolded protein response, UPR) is turned on and that UBX2/SEL1 transcription is up-regulated in response to ER-stress. In addition, we have shown that Ubx2/Sel1p function is not limited to ERAD substrates. It is also involved in the breakdown of cytosolic substrates that are processed by membrane-bound ubiquitin ligases.

Ubx2/Sel1p belongs to the family of UBX-domain proteins, which have been shown to interact directly with Cdc48p. Yeast Ubx2/Sel1p is unique since it comprises not only an UBX domain but an ubiquitin-binding domain (UBA) in addition. Both domains are separated by two transmembrane segments which anchor Ubx2/Sel1p in the ER-membrane. Based on our experimental data, we propose that Ubx2/Sel1p acts upstream of the Cdc48p ATPase complex in the degradation of substrates of the ubiquitin proteasome pathway in general. Furthermore, we have provided evidence that the UBA-domain of Ubx2/Sel1p may be specifically required for the release of ERAD substrates from the membrane but not for degradation of cytosolic substrates. Ubiquitin-binding domains are common motives of ERAD components. Thus, it is tempting to speculate that ERAD substrates are guided from a dislocation channel to the proteasome by a cascade of ubiquitin-binding factors, including Ubx2/Sel1p. These successive interactions may protect the substrate from premature deubiquitination and, in addition, contribute to the directionality of the transport process.

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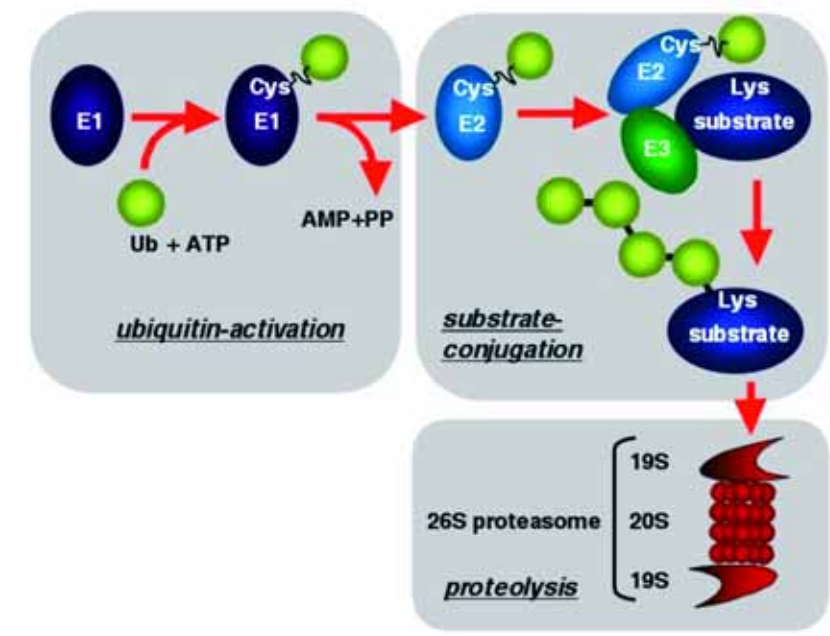

Figure 1. The ubiquitin-proteasome system (UPS). A cascade of enzymatic reactions leads to ubiquitination of lysine residues of the substrate. First, the ubiquitin-activating enzyme (E1) hydrolyses ATP and forms a high-energy thioester linkage between its active site cysteine and the C-terminus of ubiquitin (Ub). Activated ubiquitin is then transferred to a member of the family of ubiquitin conjugating enzymes (Ubc or E2). E2 enzymes together with ubiquitin protein ligases (E3) attach ubiquitin to lysine residues of substrate proteins. In most cases, E3s function as substrate binding factors that align the substrate and E2 in a way that facilitates ubiquitination. However, one class of E3s containing the HECT (homology to E6AP C-terminus) domain is able to form a thioester intermediate with ubiquitin. In this case, direct contact between E2 and substrate (as depicted in this figure) is not necessary. A polyubiquitin chain is formed on the substrate by the successive addition of ubiquitin molecules to lysine residues of the previously attached ubiquitin. Polyubiquitinated proteins are recognized by specific subunits in the 19S capping complexes of the 26S proteasome. The AAA-type ATPases in the 19S cap are required to feed the polypeptide chain into the central chamber of the 20S core particle that harbors the proteolytically active sites. Deubiquitinating enzymes that are associated with the 19S cap cleave ubiquitin from the substrates before the terminal digestion. (See also Meusser, B., Hirsch, C., Jarosch, E., and Sommer, T. (2005) ERAD: The long road to destruction. Nature Cell Biol. 7, 766-772)

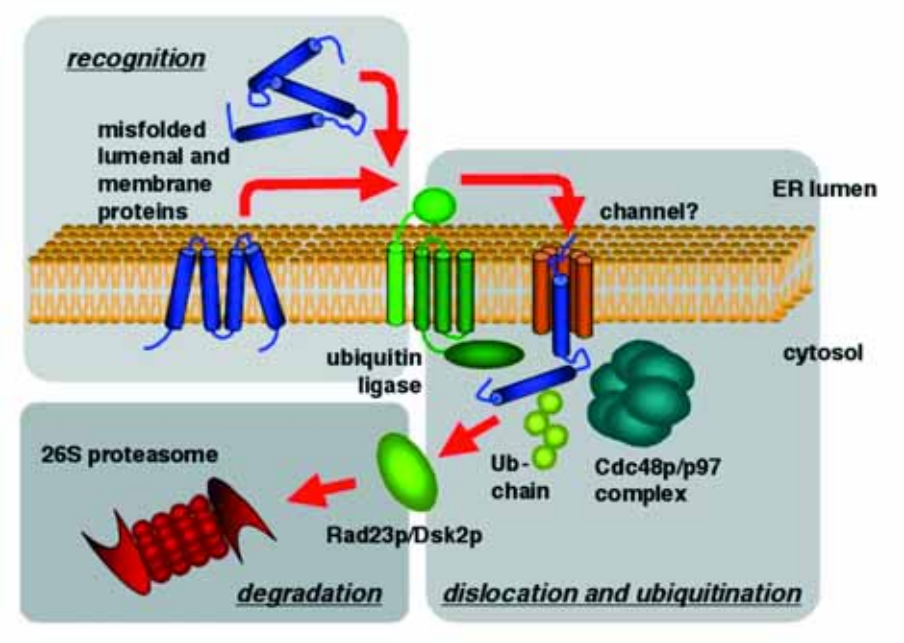

Figure 2. Proteasomal degradation of ERAD targets. Aberrant proteins are recognized within the ER lumen by quality control mechanisms which escort terminally misfolded polypeptides to a putative channel that facilitates their export from the ER. Cytoplasmically exposed lysine residues are ubiquitinated by ubiquitin ligases. Dislocation is completed with the help of the Cdc48p/p97 complex and membrane-extracted substrates are conveyed to the proteasome by accessory factors such as Rad23p and Dsk2p. (See also Meusser, B., Hirsch, C., Jarosch, E., and Sommer, T. (2005) ERAD: The long road to destruction. Nature Cell Biol. 7, 766-772)

Neuber, O., Jarosch, E., Volkwein, C., Walter, J., and Sommer, T. (2005) Ubx2/Sel1p links the Cdc48p/p97-Complex to Endoplasmic Reticulum Associated Protein Degradation. *Nature Cell Biol.*, in press.

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Regulation of Nuclear Transport Processes

Katrin Stade
(Helmholtz Fellow)

The transport of macromolecules between the nucleus and cytoplasm is a major cellular activity with respect to both the number of particles involved and energy consumption. Complex processes, such as signal transduction and cell cycle progression, which also rely on nuclear transport reactions are tightly regulated at this level to occur efficiently and in a coordinated fashion. Not surprisingly, mutations in components of the nuclear transport machinery result in deregulation of these processes and may ultimately lead to the development of severe human disease such as cancer, primary billiary cirrhosis, and triple A syndrome.

Post-translational modifications, such as phosphorylation, are well known to control nuclear transport processes. More recently, a novel protein modification system has been proposed to play a role in nucleocytoplasmic trafficking. The ubiquitin-like small modifier SUMO which previously had been shown to play an important role in transcriptional repression, chromosome segregation, and DNA repair, was also recognized as a key player for one particular nuclear protein import pathway. Using a genetic screen in the buddding yeast, Saccharomyces cerevisiae, we have recently identified several novel factors involved in nuclear transport reactions and gene silencing. Using in vivo experiments as well as in vitro assays, we are currently studying these genes in more detail in order to elucidate the functional implications of SUMO modification for the corresponding proteins.

Another research interest of the lab is the karyopherin Crm1/Xpo1 which, in virally infected eukaryotic cells, is responsible for the nuclear export of the HIV pre-messenger RNA particle. The budding yeast orthologue Xpo1 was found to export reporter proteins containing a nuclear export signal and several classes of cellular RNA. Since then, Xpo1 has been characterized in greater detail but many questions still remain: What are the major cellular export substrates for this exportin and how are they transported to the cytoplasm? Does Xpo1, like other components of the nuclear transport machinery, play an additional role in chromosome segregation during mitosis and how is this achieved? We are currently addressing these questions by implementing genetic strategies as well as biochemical methods in the budding yeast Saccharomyces cerevisae.

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Post-Translational Modifications

Gunnar Dittmar

Regulation of protein function is a central theme in cellular control. Several mechanisms for the post-translational modification of proteins with other molecules have been identified and studied in detail. This includes the modification with small molecules (phosporylation or methylation) as well as the modification with small proteins. The most prominent example for such a modification is the polypeptide ubiquitin. Recently, a number of proteins have been identified which share a great structural similarity to ubiquitin. Most of them are also covalently linked to their substrate proteins using an enzymatic cascade. In contrast to ubiquitin, none of them seems to act in protein degradation but rather they appear to modulate protein function in terms of activity or localization. The focus of our current work lies in the functional analysis of the small protein Hub1. This protein shows an ubiquitin-like structure and has a stunningly high evolutionary conservation. In contrast to its relatives, Hub1 does not appear to be covalently conjugated to other proteins but, rather, forms incredibly stable, non-covalent adducts.

Hub1 is involved in a number of cellular processes, including cellular morphogenesis and mRNA splicing. We were able to show that Hub1 interacts with components of the polarisome. This complex is a regulator of cell morphogenesis. It interacts with Cdc42 and Cdc24 two components, which control the selection of an emerging new cell. If Hub1 is removed from the cell, this process is disturbed and the cell fails to perform in the correct way. In addition, we were able to unravel the involvement of Hub1 in mRNA splicing. Here, we were able to show that *hub1* deletion mutants are not able to import a component of the splicing complex into the nucleus of the cell. This leads to an accumulation of unspliced pre-mRNAs in the cell and, ultimately, to cell death.

Currently, we are interested in the function which Hub1 takes in the regulation of the polarisome complex. Here, we are investigating whether Hub1 acts as a localization factor or as a modular surface for the association of the components of the complex. Furthermore, we are also identifying new targets for Hub1 using genetic and protein-biochemical approaches.

Selected Publications

Sommer, T. and Dittmar, G. (2005) Ubiquitination. Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine. Springer (in press).

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Patent Applications

Method for the analysis of multiple sequence alignments of related protein sequences, a computer program, computer device and a computer readable medium thereof
(patent pending).

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Control of DNA Replication

Manfred Gossen

The research group is interested in the mechanisms controlling the initiation of DNA replication in multicellular eukaryotes. In metazoans, the interplay between chromosomal *cis* elements and the *trans* acting factors (initiators) that contribute to the initiation of replication is poorly analyzed. This, however, would be a prerequisite for a detailed understanding of the processes controlling genome duplication and cellular proliferation. Current approaches in our laboratory include the analysis of proteins forming the prereplicative complex (preRC), in particular the Origin Recognition Complex (ORC), the eukaryotic initiator protein. Currently, both *Drosophila* and mammalian tissue cultures are used as experimental systems.

In addition to the above topics, the group is also interested in transcriptional cross-talk between neighboring transgenes as well as the long-term stability and homogeneity of their expression patterns.

Localization and cell cycle dynamics of the Drosophila ORC

ORC is likely to function as the initiator protein in eukaryotes, i. e. its binding to chromosomal sites specifies the origins of bi-directional DNA replication. These sites are only poorly characterized in metazoan organisms. *Drosophila melanogaster* offers several distinct advantages for the analysis of replication initiation proteins. Among them are the availability of a large number of hypomorphic alleles of replication initiation genes and an embryonic development which relies on maternally supplied stockpiles of replication factors. To analyze the specificity of ORC DNA binding in vivo, we generated GFP fusion constructs with the gene for one of the subunits, Orc2, in its authentic chromosomal environment. This construct was expressed in transgenic flies in an Orc2-null background. This approach allows us to determine the subcellular localization of ORC throughout the cell cycle, in particular its chromatin association (with the help of complementary histone-RFP fusions), and reveals changes in the dynamic behavior of this protein complex in different tissues and throughout development. We are currently addressing the question if this experimental setting can also be used to unravel the molecular mechanisms behind the cell cycle regulated ORC redistribution and are also trying to visualize the location and activity of specialized individual replication origins, the chorion gene amplification sites in follicle cells.

Biochemical characterization of the human ORC

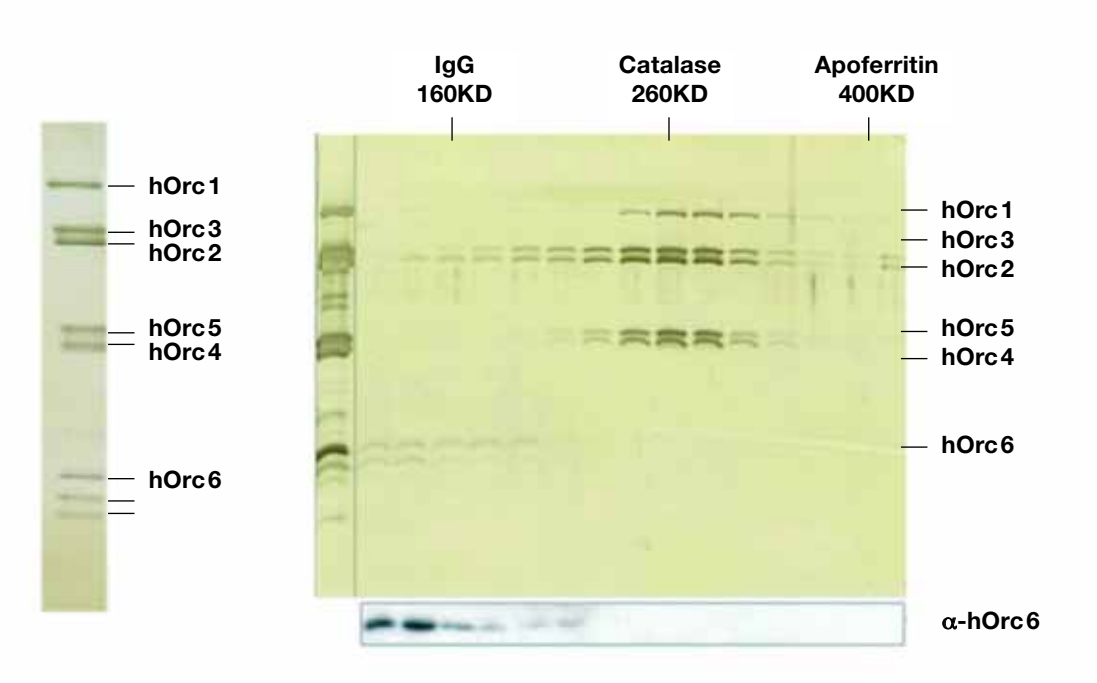
We were able to co-express genes for all six subunits of human ORC in insect cells and to purify the resulting protein complex to homogeneity. Using a *Xenopus in vitro* replication assay, the functionality of the recombinant ORC was demonstrated. It turns out that human ORC is capable of forming various distinct sub-complexes, which differ in their stability and DNA binding properties. By omitting individual subunits in this protocol, specific interactions among the ORC proteins were revealed. According to the *Saccharomyces cerevisiae* paradigm, ORC's binding to DNA is expected to be by ATP dependent. We are currently investigating if this unusual mode of regulating sequence specific DNA interactions is connected to the ATP dependence of specific subunit interactions that we observed in our *in vitro* assays. To this end, we are also testing the biochemical properties of recombinant human ORC defective in ATP interactions. The goal of these studies is a better understanding of how homologous initiator proteins like those of yeast and humans accomplish highly divergent modes of origin determination.

Ablation of preRC proteins

The genomes of all eukaryotic organisms code for six different MCM proteins which can interact with each other and show helicase activity, possibly constituting the eukaryotic replicative DNA helicase. The other subunits apparently suppress or regulate the helicase activity. In a colloboration with atugen AG, Berlin, we investigated the effects of special antisense molecules ("Geneblocs") directed against one of the MCM genes in primary human fibroblasts. The knockdown of this gene results in the growth arrest of the cells and of DNA synthesis as well as an increase in the G2/M population of the transfected cells. Together with atugen, we are currently evaluating the use of this technology as well as alternative RNAi protocols for the control of cell proliferation *in vitro* and *in vivo*.

Interference and epigenetic regulation of transgenes

Transcription units placed randomly in the chromosomes of mammalian cells are subject to both epigenetic control and the influence of nearby transcription signals. These findings have important implications for the design of gene expression vectors for transgenesis and gene therapeutic approaches. Frequently, however, it is desirable to transfer more than one transcription unit in one step. We are analyzing the effects these transgenes exert on each other by using an inducible transcription system. Upon induction of a target gene, a neigh-



Recombinant human ORC. The six human ORC genes were expressed by co-infecting Sf9 cells with respective baculovirus constructs. The left panel shows the recombinant human ORC after affinity purification. Glycerol gradient analysis (right panel) shows the homogeneity of this material aside from the smallest subunit, hORC6, which dissociates from the complex under this experimental conditions.

boring, "constitutive" transcription unit can be co-regulated depending on the nature of the promoters used. Vice versa, these promoters can have a dominant influence over the characteristics of the inducible transcription unit. These effects are furthermore analyzed taking into account the effects of epigenetic transgene control like DNA methylation and chromatin compaction. We recently established protocols allowing for the reproducible generation of high level expressing stable cell lines that can escape epigenetic downregulation over prolonged periods of time.

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Debs, P, Bonin, A, Gossen, M. (2005) Minichromosome maintenance proteins are required for passage through S and M phase in primary human cells. submitted

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Nuclear Signalling and Chromosome Structure

Harald Saumweber

Communication between cells is essential for development, the control of proliferation, and the maintenance of the differentiated state. In the nucleus, signalling molecules alter the expression state of genes in their nuclear chromatin environment by interaction with regulatory factors and chromatin modifying enzyme machineries already in place. Malfunction of these processes causes a number of severe human diseases, most prominent among them are different types of human tumors. In recent years, significant progress in the understanding of gene control mechanisms was obtained by the study of human cells and using the mouse as a model system. This was built upon observations made in genetically tractable organisms like yeast, worms, and flies, based upon the fact that many of the crucial events are molecularly conserved in evolution. In these animals, the advantage of performing classical genetics on genomes of low complexity and the availability of simple methods to obtain transgenic animals for cell and tissue specific manipulation of genes is still indisputable. The results obtained were and will be translated into a deeper understanding of human development and disease. Using *Drosophila* as a model our group starting in July 2004, we have been investigating chromatin switches crucial for Notch and TGF-β signal transduction and the molecular architecture of chromosomal domains important for the co-regulation of target genes.

Target gene regulation in the Notch pathway

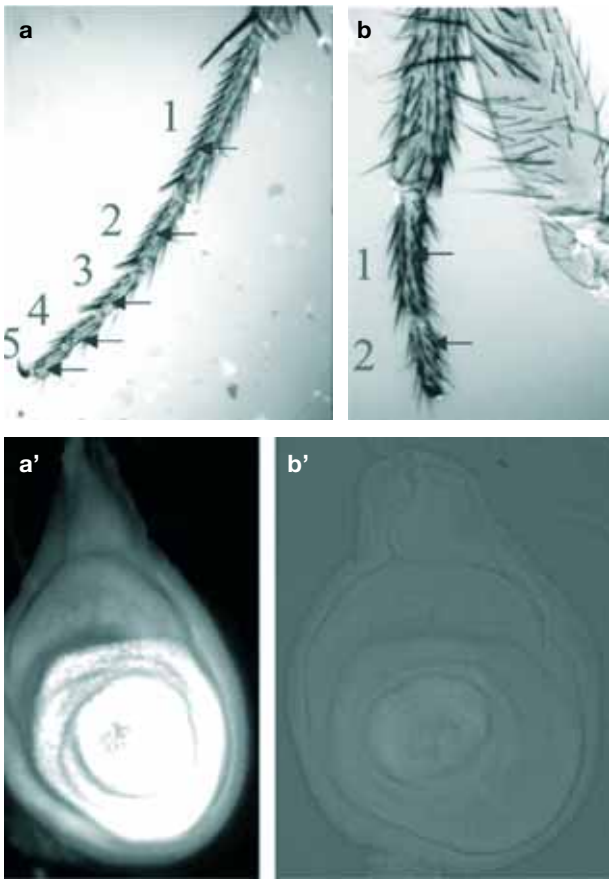
The Notch gene was first identified in *Drosophila* by its dominant phenotype of inducing notches at the wing margin of flies. Later, it was found that the Notch signalling pathway in the fly contributes to cell fate decision and differentiation of numerous tissues as in the nervous system, the eye, the appendages, and the muscle tissue. In humans, there are several Notch homologous genes that are involved in epithelial, neural, and muscle development and in hematopoiesis. Inappropriate activation of the pathway results in a number of diseases such as some forms of leukemia. The Notch transmembrane receptor, upon interaction with specific ligands on the surface of adjacent cells, splits off an intracellular domain which migrates into the nucleus and targets an activator complex (including histone acetyltransferase) to genes that previously were repressed by a silencing complex (including SMRT, Sin3a and histone deacetylases) bound to a chromatin platform provided by proteins like Su(H)/CBF1. 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 coregulator protein. The mammalian homologue was shown to interact with nuclear components of the Notch pathway as was shown by our group in *Drosophila*. We demonstrated that this interaction is biologically important since the expression of several Notch target genes is dependent upon the presence of Bx42/SKIP and tissue specific knock out of Bx42/SKIP results in *Notch*-like phenotypes. Currently, we are dissecting the molecular contribution of this protein to the switching process in Notch dependent gene activation and repression (D. Negeri, S. Lehmann).

Control of cell proliferation by Notch signalling

Besides contributing to cell fate decisions and differentiation, Notch signalling is involved in the control of cell proliferation in a process that also may require Bx42/SKIP. Phenotypically, loss of Bx42 expression results in eye/antennal transformation and loss of eye and head structures resulting from proliferation defects in the eye imaginal disc. We will investigate whether this is mediated by misexpression of Notch target genes like *eyegone* and downstream genes. However, Bx42/SKIP also interacts with and counteracts the repressive effect of the Rb protein and shows an interaction with the E2F family of cell cycle regulators. The effect of available dominant negative mutations on the expression and activity of cell cycle regulators will be tested and phenotypic consequences will be analyzed. By cell and tissue specific expression of available Bx42 dominant negative mutations, the effect on downstream cell cycle regulators shall be tested on cDNA microarrays (D. Negeri in collaboration with Dr. A. Klebes genetics department FU Berlin).

Target gene regulation in the TGF-β/dpp pathway

The TGF-ß (BMP, activin) pathway is involved in many steps in cell fate decision, differentiation, and proliferation in mammalian development. In *Drosophila*, TGF-β signalling is restricted in complexity and is well known for the Dpp-pathway. Bx42/SKIP is involved in this pathway as well, since its reduction results in a number of *dpp*-like phenotypes. We could show that downregulation of Bx42 results in loss of or altered expression of dpp-target genes in leg and wing imaginal discs. Furthermore, Bx42 directly interacts with the *Drosophila* Smad proteins dSmad2, Mad, and Medea. This interaction is of biological importance since phenotypically the downregulation of Bx42 can be complemented by simultaneous overexpression of Medea. As in Notch signalling, Bx42/SKIP may mediate its effects by bridging transcription regulators with chromatin modifiers. The molecular contribution of this pro-



a. normal *Drosophila* leg with 5 tarsal segments; **b.** Only the proximal 2 tarsal segments remain following Bx42 knock down in leg imaginal discs; **a´.** Expression of the Dpp target gene *distal-less* (LacZ reporter) in wild type leg discs; **b´** loss of expression following Bx42 knock down.

tein to Dpp-dependent gene activation shall be elucidated (M. El Hachoumi PhD thesis in progress).

Chromatin domains and boundaries

Transgenes inserted into the mammalian genome regularly become silenced at their site of insertion. Interestingly, there exist certain chromosomal boundary elements that shield for this silencing by providing enhancer/silencer blockers or barrier elements that block the spreading of adjacent heterochromatin. Formation of boundaries supports the concept that eukaryotic genomes are organized into functionally independent chromosomal domains. At present, the best known chromosomal domain is the globin domain in human, mouse, and chicken. In addition to scientific reasons, knowledge of chromosomal domain structure and function is of immediate medical importance, in particular in the case of gene therapy. In *Drosophila*, conserved stretches of chromosomes differing in degree of condensation become apparent by the structure of polytene chromosomes (dark bands and light interbands) suggesting a chromosomal domain organisation separated by boundaries. By isolating and characterizing interband DNA sequences (where boundaries may be formed), associated proteins, and histone modifications, we try to understand the process of chromosomal domain formation with special emphasis on chromosomal boundaries. Mutation of an interband specific zinc finger protein found by our approach results in dramatic loss of chromosomal structure, presumably by affecting the maintenance of chromosomal boundaries. A part of the project performed at the MDC investigates a newly discovered interband specific chromodomain protein, its mode of chromosomal binding, and its role in the formation and maintenance of chromosomal domains (M. Gan PhD student of the international PhD program).

Selected Publications

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Evolution, Regulation and Genetic Applications of Transposable Elements in Vertebrates

Zoltán Ivics

Project Description

Work in the "Transposition" group involves transposable DNA elements. We follow two main areas of research: 1) Molecular biology of DNA transposition in vertebrate cells and 2) Applications of transposable elements in vertebrate genetics. During the past years, we have laid the foundation for using Sleeping Beauty (SB) and Frog Prince (FP) as molecular tools to address both of these areas. In search for host-encoded factors that regulate the transposition reaction, we identified the DNA-bending protein HMGB1 and the DNA repair protein Ku70 as cofactors of SB transposition. HMGB1 most likely plays a role by assisting synaptic complex formation during transposition, whereas Ku70 is required to repair the double-stranded DNA gaps after transposon excision. We have screened a human gene library using the yeast two-hybrid technology and identified the Miz-1 protein as an interactor of the SB transposase. Miz-1 is a transcription factor of several cell-cycle regulatory genes, including cyclin D1. SB transposase downregulates cyclin D1 expression in a Miz-1-dependent manner, and induces a temporary delay in the cell-cycle at the G1 phase, where the nonhomologous end joining pathway of DNA repair is most active. On the front of vector development for insertional mutagenesis and gene therapy, we developed improved SB-based vectors that show an almost 10-fold increase in transpositional efficiency when compared to the first-generation vectors. We have shown unprecedented gene-trapping efficiencies with FP transposition in mammalian cells, thereby demonstrating the usefulness of this vector system for functional gene analyses in vertebrate species. We established a useful and efficient, transposon-based vector system for the generation of stable RNAi knockdown cell lines. We are currently concentrating our efforts on the following projects:

1. SB and FP have a number of advantages as gene vectors when compared to current viral and non-viral gene transfer technologies. Our goal is to evaluate, develop, and modify the SB and FP vector systems so that they will become efficient and safe vectors for human gene therapy. Specifically, we are in the process of establishing methodologies that allow targeted transgene insertion into predetermined sites or regions in chromosomes. Targeted transposition could be a powerful method for safe transgene integration in human applications and for target-selected gene knockouts in vertebrate models.

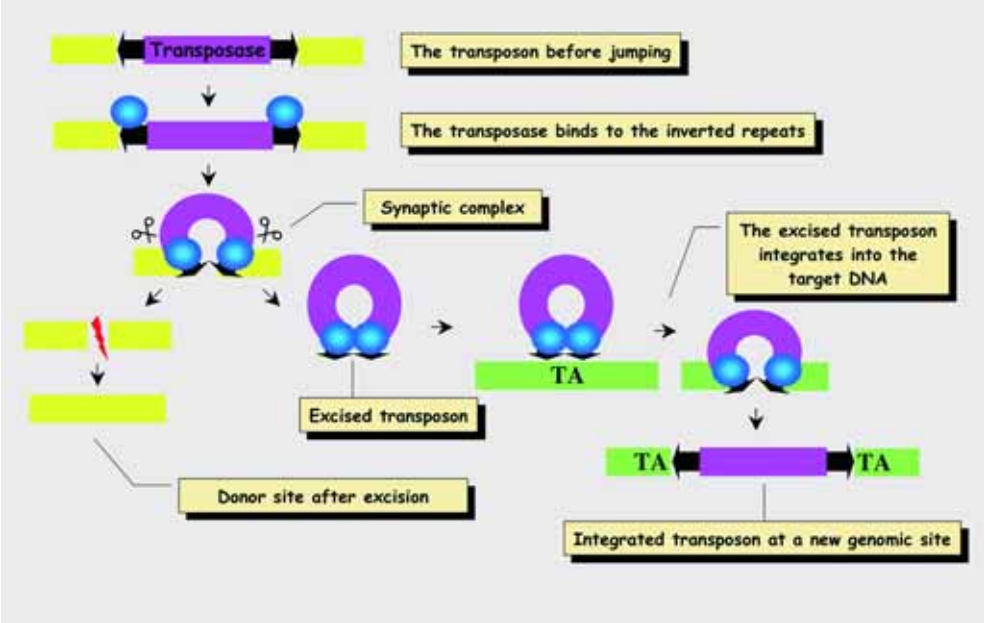
2. We exploit transposons to determine the identity, function and biological relevance of genes that are associated with vertebrate embryonic development and human disease, by isolating their counterparts from model organisms such as fish, frogs, and mice. Specifically, we are in the process of:
   a) introducing both directed and random mutations into the SB and FP transposase genes in the hope that we can derive hyperactive versions of the transposon systems. With such hyperactive vectors, we hope to be able to efficiently knock out genes in vertebrate model organisms;
   b) conducting an FP transposon-based insertional mutagenesis screen in the zebrafish, using gene-trap transposon vectors, whose expression is dependent on transposition into transcribed genes. Spatial and temporal patterns of reporter expression can be co-localized with phenotypic changes in developing zebrafish embryos;
   c) conducting SB- and FP transposition-based gene trapping in mouse embryonic stem cells. We hope to be able to complement existing insertion site profiles obtained with retrovirus- and plasmid-based vector with new transposon vectors resulting in an overlapping, but different set of target genes;
   d) setting up an insertional mutagenesis screen in the mouse *in vivo*, with the goal of generating a series of mutations in the syntenic region of the Williams Beuren syndrome locus. We hope to be able to uncover the genetic basis of this disease using this mutagenesis approach.

3. We are investigating the molecular interactions between the transposon and the host cell.
   We continue our search for cellular interactors and regulators of DNA transposition in vertebrate cells. We are taking an approach of *in vivo* labelling of the SB transposase and subsequent affinity purification from cellular extracts. In search for gene regulatory networks that are activated in response to transposition, we are in the process of identifying relevant transcriptional changes of gene expression by using Affymetrix gene chips. This approach allows us to gain insight into the complex regulation of transposition in vertebrate cells.

Selected Publications

Kaufman, C.D., Izsvák, Z., Katzer, A. and Ivics, Z. (2005). *Frog Prince* transposon-based RNAi vectors mediate efficient gene knockdown in human cells. *Journal of RNAi and Gene Silencing* 1:97-104.

Izsvák, Zs., Stüwe, E.E., Fiedler, D., Katzer, A., Jeggo, P.A. and Ivics, Z. (2004). Healing the wounds inflicted by *Sleeping Beauty* transposition by double-strand break repair in mammalian somatic cells. *Mol. Cell* 13:279-290.



Cut-and-paste DNA transposition. The transposon consists of terminal inverted repeats (black arrows) flanking a gene encoding the transposase protein. Transposition is initiated by binding of the transposase (blue dots) to binding sites located within the terminal inverted repeats of the element. The two ends of the element are then probably paired through interactions of transposase subunits, thereby forming a synaptic complex. Excision of the transposon from the donor site likely occurs in the context of the synaptic complex. The transposon is physically removed from the donor site by cuts at the transposon ends. Cellular DNA repair factors will seal the gap after transposon excision. The transposition process is completed after reintegration of the element into a TA target site, which gets duplicated. The TA target site duplications flank the newly integrated transposon.

Izsvák, Zs. and Ivics, Z. (2004). *Sleeping Beauty* transposition: Biology and applications for molecular therapy. *Mol. Ther*. 9:147-156.

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Miskey, Cs., Izsvák, Zs., Plasterk, R.H. and Ivics, Z. (2003). The *Frog Prince:* a reconstructed transposon from Rana pipiens with high activity in vertebrates. *Nucleic Acids Res.* 31:6873-6881.

Patent Applications

1. The Frog Prince: a transposon vector for genetic transformation of vertebrates (MDC/NKI)
2. Transposon-based targeting system (MDC)
3. Reconstructed human mariner transposon capable of stable gene transfer into chromosomes in vertebrates (MDC)

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Mobile DNA Elements in Vertebrates

Zsuzsanna Izsvák

Project Description

The “Mobile DNA” group focuses on transposable elements. Transposons are non-viral, mobile genetic elements with the ability to move from one location to another inside genomes. Transposons are on their way to being developed as indispensable tools for somatic and germ-line mutagenesis and gene-delivery, including gene therapy.

By the mid 1990’s, Tc1/mariner-like elements had been isolated from several vertebrate species but nearly all-native elements were found inactive in transposition due to the presence of mutations in the coding region of the transposase. This lack of active vertebrate DNA transposons limited the ability to manipulate vertebrate genomes. This situation has changed by the “resurrection” of two ancient vertebrate transposons, Sleeping Beauty (SB) and Frog Prince (FP). I have been involved in establishing both of these transposon systems.

Sleeping Beauty and Frog Prince DNA transposons are members of the Tc1/mariner family. SB an FP elements encode a transposase protein necessary and sufficient for transposition. The transposase coding sequence is flanked by a pair of terminal inverted repeats containing transposase binding sites. SB/FP is typically used as a two-component system: 1) a gutted transposon carrying a reporter gene(s), flanked by the inverted repeats, and 2) the transposase expressed under the control of a heterologous promoter. Both SB and FP have been shown to transpose efficiently in a variety of vertebrate cell lines. SB was demonstrated to transpose in mouse somatic tissues, stem cells and in the germ line.

In less than a decade, our laboratory and others have successfully adapted the SB system to several major areas of vertebrate genomics applications, as germ line transgenesis, somatic transgenesis (gene therapy), germ line insertional mutagenesis, and somatic cell mutagenesis. Since its debut in 1997, there has been ongoing engineering of SB, including mutations in the transposase binding sites and searching for ever more active versions of SB transposase. The stakes for optimization are high, as even a 2-fold increase in activity could translate into a significant improvement, e.g. on gene therapy efficacy or mutagenesis efficiency. Our efforts in developing a more active transposon vector system resulted in a 5-fold (FP) and 10-fold (SB) increase in transposition frequency compared to the original versions.

The transposition process is host dependent and three factors were identified in the last few years that are involved in SB transposition. HMGB1, a bending protein is involved in the synaptic complex formation of SB transposition. A repair factor, Ku70/80 of the DNA double strand break repair has a role in repairing the double strand breaks generated by transposon excision. The Ku-factors seem to be involved in the regulation of transposition as well. The association of Miz-1 with SB transposition indicates a possible coordination of SB transposition with cell cycle control.

The research in the Mobile DNA group is focusing on the two main areas:

1) Mechanism of transposition
2) Develop and apply innovative, transposon-based technologies for functional genomics in vertebrate models and for human gene therapy.

Identification of host repair factors involved in transposition. Several factors determine which DNA repair pathways are used to seal the gaps after a transposition event, including the transposon itself, the structure of the lesion, the host organism, the status of the cell cycle, and whether transposition occurs in the soma or in the germline. My work has established that NHEJ and HR both contribute to the repair of SB-induced DSBs in mammalian somatic cells. Our project is aimed at surveying different cellular factors involved in DNA replication, repair, and damage signaling for their potential roles in SB transposition. Cellular mechanisms that are directly involved in repairing transposition-inflicted DNA lesions or can attenuate the damage should have crucial roles in establishing stable host-transposon co-existence. The pathways and factors our group proposes to study include DSB repair (XRCC2/3 in HR and DNA-PK in NHEJ); DNA damage signaling, (ATM and ATR); processing of recombination intermediates formed during DNA replication (BLM helicase); postreplication repair of DNA damage-induced replication arrest when DNA lesions left unrepaired prior to the initiation of S phase (Rad6/Rad18) and mismatch repair (Msh2/Msh6/Mlh1).

Cell-cycle regulation of SB transposition. Infection by oncoretroviruses such as Murine Leukemia Virus (MLV) and Rous Sarcoma Virus (RSV) requires cell division. In nondividing cells, viral entry, uncoating, DNA synthesis, and formation of viral preintegration complexes (PICs) occur at the same rate as in dividing cells, but integration fails to occur. It has been thought that the physical size of the PIC exceeds the upper limit for passive diffusion through nuclear pores but, during mitosis, the nuclear membrane disassembles, rendering the chromosomes accessible to the PIC. However, imposed nuclear translocation of MLV DNA in nondividing cells is not sufficient for stable transduction. It appears, therefore, that additional cellular factors activated during S phase or DNA repair synthesis are required for efficient retroviral

integration. Because SB is being developed as a gene therapy vector, it is of critical importance to establish whether transposition can efficiently occur in non-dividing cell types (most target tissues in vivo are non-dividing). Our plan is to investigate whether *Sleeping Beauty* requires active cell division for transposition and, if yes, whether transposition can occur throughout the cell cycle or whether it is restricted to certain cell-cycle phases.

Isolating hyperactive transposase versions by directed evolution. It is widely believed that naturally occurring transposons have not been selected for the highest possible activity and are strongly down-regulated. Since transposons co-exist with their hosts, transposition activity is regulated in order to avoid insertional inactivation of essential genes. Low intrinsic activity, self-regulation, and interaction with cellular host factors appear to allow wild type transposons to persist in the host without producing serious levels of genetic damage. The *Sleeping Beauty* (SB) and *Frog Prince* (FP) transposons show efficient transposition in cells of a wide range of vertebrates, including humans. Yet, these transposons are not expected to have the highest possible activity, therefore they would be excellent candidates for *in vitro*, directed evolution. Our group proposes to establish and conduct a large scale genetic screen to derive hyperactive transposases generated by *in vitro* evolution. In addition, our group is establishing both an *in vitro* assay system for the Sleeping Beauty tranposition and, in collaboration with the team of Norbert Hübner, an *in vivo* assay for a rat endogenous retrovirus responsible for a recent phenotypic mutation.

Develop and apply innovative, transposon-based technologies for functional genomics in vertebrate models and for human gene therapy. DNA-based transposons are natural gene delivery vehicles. Similarly to retroviruses, these elements integrate into the chromosomes of host cells but their integration mechanism does not involve reverse transcription and they are not infectious. Since transposase-deficient elements can be mobilized if the transposase is provided *in trans*, it is possible to stably integrate a desired DNA molecule into the vertebrate genome. These features make transposons ideal for the development as molecular tools for applications such as transgenesis, gene therapy, and functional genomics.
Our objective is to develop non-viral, recombinase-based technologies for ex vivo gene therapeutic applications as an alternative of current viral/non-viral gene delivery technologies with the aim of circumvent the toxicity and immunogenicity problems raised by viral delivery systems.

Insertional mutagenesis. Transposons can be harnessed as vehicles for introducing genetic mutations into genomes. The genes inactivated by transposon insertion are “tagged” by the transposable element, which can be used for subsequent cloning of the mutated allele. The first mutagenesis screens have established that SB can generate a high number of mutations in mouse germinal cell. It is possible to generate mice that carry an integrated transposon in every second or third stem cell in the testis, suggesting the feasibility of using SB for insertional mutagenesis in mice. SB has a preference (~70%) to jump locally (i.e., close to the donor locus), referred to as “local hopping”, and most of the local transpositions are clustered within a 2 to 3 Mb region. This feature makes SB integration suitable to generate mutations in a particular locus of interest. *Sleeping Beauty* in its present form is being probed in insertional mutagenesis screens in vertebrates. Our goal is to establish insertional mutagenesis screens in rat using hyperactive transposases. Rats are important models for cardiovascular diseases. The cardiovascular community would greatly benefit from the establishment of a powerful genomic tool. The local hopping phenomenon will be exploited to generate rat mutants in chromosomal regions, where genes of interest form gene clusters QTLs. Hyperactive transposase versions will also be used to develop efficient transposons for insertional loss- and gain-of-function mutagenesis of rat genes, with the option of conditional reversion of mutant phenotypes. This work will be done in collaboration with Norbert Hübner’s group.

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Zayed, H., Izsvák, Zs., Khare, D., Heinemann, U. and Ivics, Z. (2003). The DNA-bending protein HMGB1 is a cellular cofactor of Sleeping Beauty transposition. Nucleic Acids Res. 31:2313-2322.

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Christopher D. Kaufman, Zsuzsanna Izsvák, Andrea Katzer and Zoltán Ivics (2005). Frog Prince transposon-based RNAi vectors mediate efficient gene knockdown in human cells Journal of RNAi and Gene Silencing.

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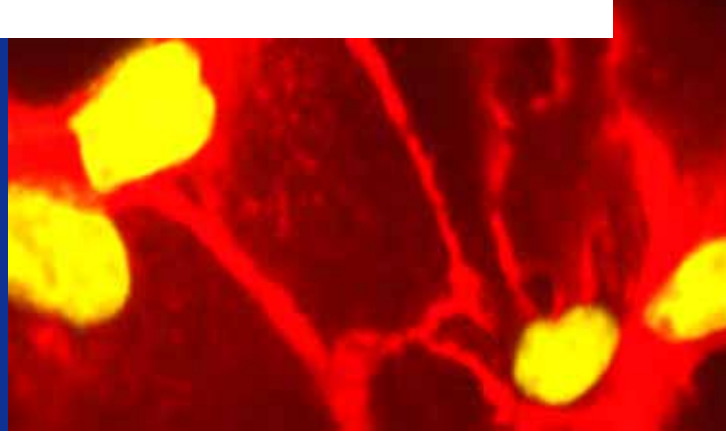

Macromolecular Structure and Interaction

Udo Heinemann

Many biological processes can be studied at the atomic level where macromolecular structures and interactions may be analyzed in exquisite detail. These studies are usually based on X-ray crystallography, an exceptionally powerful tool to study the structures of proteins, nucleic acids, and their complexes. It enables the determination of the precise arrangement of all atoms and the shape and property of all surfaces in single molecules as well as in huge molecular complexes. In our laboratory, crystallographic analysis is combined with biochemical and biophysical experiments to explain biological functions and observe specific ligand binding to proteins. The complementary technique of NMR spectroscopy, also available on the Berlin-Buch research campus, is employed to determine structures of proteins that do not crystallize and to address specific issues of protein dynamics or ligand binding. To prepare protein samples for crystal structure analysis, we rely on techniques and resources recently developed in a structural proteomics context. Within the Berlin-based Protein Structure Factory, more than 500 protein-coding human genes were cloned into expression vectors and several three-dimensional structures of human proteins were recently determined, serving as starting points for further studies. We are primarily interested in proteins interacting with nucleic acids, involved in vesicle transport, or linked to disease states.

Nucleic acid-interacting proteins

The sequence-specific binding of proteins to DNA follows structural patterns that are fairly well understood. The best known of these patterns is the interaction of helix-turn-helix (HTH) proteins with the major groove of DNA double strands. HTH motifs occur in DNA-binding proteins from all kingdoms of life. In virtually all cases where they have been observed, the sidechains of amino-acid residues anchored in the HTH helices provide for DNA sequence readout by forming hydrogen-bonded contacts with base-pair edges in the major groove. The RP4 plasmid-encoded protein KorB is a notable exception to this rule. In collaboration with E. Lanka (MPI for Molecular Genetics, Berlin), we could show that DNA sequence recognition by this protein depends on protein-DNA contacts that are mediated by residues outside the HTH motif of KorB. Although the recognition helices α4 of the dimeric KorB are aligned along two adjacent major grooves of the DNA as expected, they do not support specific protein contacts with the DNA base pairs. Instead, residues from α4 are involved in non-specific contacts with the sugar-phosphate backbone of the DNA. The residues threonine-211 and arginine-240 of KorB, which are primarily responsible for recognizing the KorB target site in the crystal as well as in solution, are located within helices α6 and α8, well removed from the HTH motif. A further peculiar feature of KorB is the protein's pronounced segmental mobility. The central, DNA-binding domain KorB-O is linked to the terminal domains KorB-N and KorB-C, the dimerization domain, via flexible peptide regions as shown by crystals structure analysis. This domain flexibility allows KorB to completely enclose a specifically bound DNA molecule. Work to further characterize the structural basis of gene regulation and partitioning of RP4, focussing on the proteins KorA and IncC, is ongoing.

The TRAPP tethering complex of vesicular transport

In all eukaryotic cells, vesicular transport is organized in a modular way. Functionally conserved sets of proteins are involved in the sequential steps of vesicle budding, uncoating, and tethering to the target membrane, as well as in membrane fusion and cargo release. One of the less well understood steps of this scheme is the recognition of a target membrane by a vesicle and its tethering to this membrane prior to the tight interaction of vesicle and target membrane-bound SNARE proteins, which ultimately results in membrane fusion. The tethering of vesicles derived from the endoplasmic reticulum to the Golgi membrane is mediated by coiled-coil proteins such as Uso1p (yeast) and the TRAPP (**tra**nsport **p**rotein **p**article)

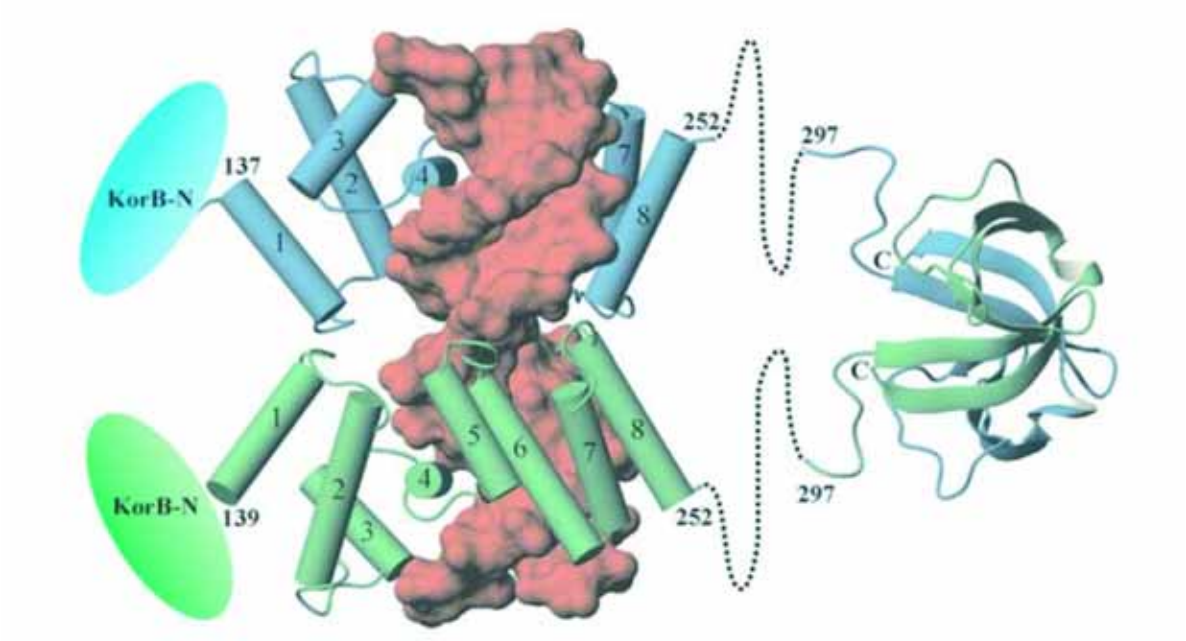

Figure 1
DNA sequence recognition by the bacteriophage RP4 KorB protein. KorB consists of three domains linked by flexible polypeptide segments. The central domain, KorB-O, is responsible for DNA sequence recognition. It contains a classical helix-turn-helix motif (α3 and α4) which plays, surprisingly, only an auxiliary role in DNA binding while the most important base-pair contacts are made by two amino-acid sidechains anchored in helices α6 and α8. Together with the KorB-N domain of unknown structure and the C-terminal dimerization domain, KorB-C, KorB-O is part of a dimeric KorB protein that completely encloses the DNA double strand in a specific complex. From Khare et al., 2004.

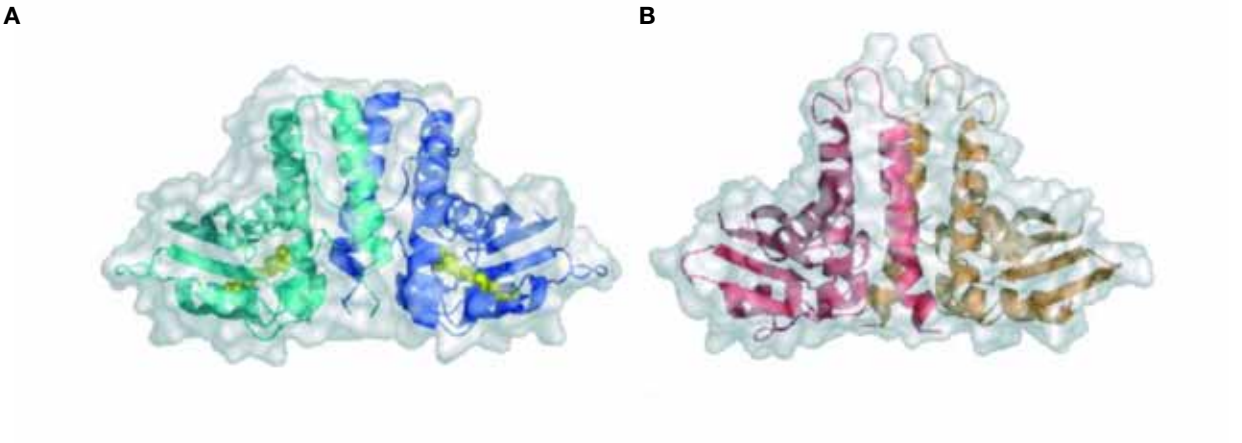

Figure 2
Crystal structures of two subunits of the human TRAPP complex involved in tethering transport vesicles to the cis-Golgi membrane. BET3 (A, Turnbull *et al.,* 2005) is dimeric in the crystal and in solution and is covalently modified by a palmitate chain linked to cysteine 68 of the protein via a thioester linkage. In spite of low sequence conservation, TPC6 (B, Kümmel *et al.*, 2005) shares a closely similar tertiary fold and dimeric arrangement with BET3. TPC6 does not contain a cysteine residue structurally corresponding to the cysteine of BET3 and is therefore not acylated. The closely similar subunit interfaces between the BET3 and TPC6 homodimers raises the possibility that BET3-TPC6 heterodimers may be formed.

complex. TRAPP I consists of 7 subunits and acts as guanine nucleotide-exchange factor (GEF) for the Rab GTPase involved in this process. A second form of the complex (TRAPP II) contains three additional subunits and plays a role in intra-Golgi vesicle trafficking.

We have recently determined the crystal structure of the human TRAPP subunit BET3 (Turnbull *et al.*, 2005). BET3 adopts an α/β-plait fold and is homodimeric both in crystals (Figure 2A) and in solution. The protein is acylated with a palmitate molecule covalently attached to the sidechain of cysteine-68 via a thioester linkage. The fatty acid chain is buried in a prominent surface cleft of BET3, preserving its solubility in aqueous buffers. Since the modified cysteine, as well as the residues lining the cleft, are conserved in BET3 molecules in cells from yeast to humans, this acylation is probably a common feature of BET3 and functionally important. Using mutated forms of human and yeast BET3 lacking the acylated cysteine, we could show, however, that the palmitoylation is neither required for membrane localization of BET3 or TRAPP nor for yeast cell viability.

The structure analysis of a second TRAPP subunit, TPC6, shows this molecule to adopt a tertiary fold and quaternary arrangement closely similar to BET3. In particular, the subunit interface region of both homodimeric proteins is very similar suggesting that they may form heterodimers in the cell. The existence of BET3-TPC6 heterodimers could indeed be proven by co-immunoprecipitation, pull-down, and chemical cross-linking experiments. Whether BET3 and TPC6 are present in the homo- or heterodimeric form inside TRAPP is very important to the overall architecture of the tethering complex whose subunits are generally assumed to be present in equimolar amounts. Interestingly, TPC6 is not acylated and possesses neither a fatty acid-binding cleft nor a cysteine residue for covalent attachment as the structurally similar BET3.

Structures of human proteins through structural proteomics

Within the Berlin-area structural proteomics consortium, the Protein Structure Factory, a large number of human genes have been subcloned into expression vectors and expressed in *E. coli* or yeast. As a preliminary result, a number of crystal structures have been determined, several of which characterize human proteins of relevance to cancer, cardiovascular disease, or general aspects of cell signaling. Among these are several molecules which will be the subject of further in-depth structural and functional studies focussing on their interactions with other proteins. Recently determined crystal structures include the product of the human aortic preferentially expressed gene-1 (APEG-1), a novel smooth-muscle differentiation marker thought to play a role in the differentiation of arterial smooth-muscle cells. APEG-1 consists of a single immunoglobulin (Ig) like domain which is shown to belong to the I (intermediate) set of Ig molecules. The protein displays a weak and salt-dependent dimerization which may be of functional importance. A further recent crystal structure reveals the fold of the human protein PTD012 located in the nucleus, the longer product of an alternatively spliced gene. PTD012 displays an $\alpha\beta\beta\alpha$ four-layer topology and contains a Zn^{2+} ion bound to three histidine sidechains at a site reminiscent of the active centers of carbonic anhydrases and various hydrolases. A search revealed ester hydrolase activity towards a synthetic substrate. The physiological substrate of PTD012 remains to be identified.

In collaboration with the group of H. Oschkinat (Institute for Molecular Pharmacology, Berlin), we are using NMR to study the dynamics and structure of proteins. The recent NMR structure determination of the C-terminal BRCT-c domain of the human breast cancer-associated protein BRCA1 shows that this domain can fold independently of a second adjacent domain, BRCT-n. However, subtle conformational differences are observed that are proposed to have an effect on the binding of phosphoserine-containing peptides.

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Computer Simulation of Biomolecular Structures, Dynamics, and Interactions

Heinz Sklenar

Computer simulations are based on physical models that describe the driving forces for the formation of molecular structures. The results lead to a better understanding of biomolecular structures in terms of their physical properties as wel as help to predict what structures are formed and how these structures interact in living systems. The computational approach complements high-resolution structure determination using X-ray crystallography and NMR spectroscopy.

During the last years, we have focused on the development of a new simulation technique based on the Monte Carlo Metropolis algorithm. The advantage of this approach, in comparison with conventional Molecular Dynamics, was shown in applications to sequence dependent DNA structures, sequence induced DNA bending, and flexible drug-DNA docking (Remo Rohs). The new algorithm has been also successfully implemented for simulating the structure and dynamics of biological membranes (Daniel Wüstner).

Monte Carlo simulation algorithm

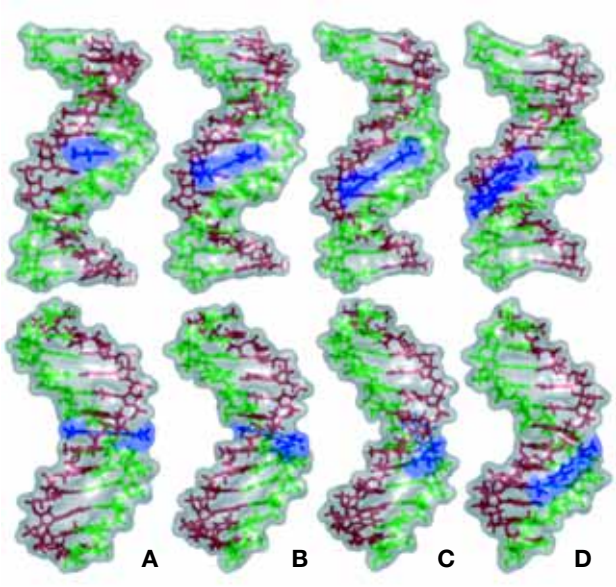
By using the constant bond lengths approximation and solving the Chain Breakage/Closure problem in the bond/torsion angle space, collective variables have been defined that maintain structural moves entirely local and allow for large conformational changes in Monte Carlo simulations. It is essentially this choice of independent variables that enables conformational equilibration on a reasonable computational time scale, which is a necessary condition for deriving meaningful structural data from the trajectories of molecular simulations. We therefore consider this algorithm as a promising basis for the development of a new generation of simulation programs for any type of biomolecular structures.

Sequence dependent structure and dynamics of DNA

Subtle sequence effects on the helical geometry and dynamics of DNA structures have been found to be critically important for selective recognition of specific base sequences by regulatory proteins. Structural libraries, derived from the analysis of experimentally determined structures and modeling results, allow for a structural description of binding sites for specific transcription factors and help in the search for sites with characteristic and common features in long sequences with unknown function. We have participated in an international initiative with the goal to improve the underlying data by using large-scale Molecular Dynamics simulations on the current state-of-the-art level. For this purpose, 39 DNA fragments, each 15 base pairs long and including all possible tetranucleotides, have been selected and simulated over 15 ns. The results, however, show that the simulation time is still too short for full conformational equilibration. Therefore, we repeated the simulations by using our new Monte Carlo (MC) technique. This work is now in progress. In case of palindromic sequences, the degree of equilibration is indicated by the differences observed for equivalent base pair steps. Compared with sequence-induced effects, such differences are already very small after 106 MC cycles, which need less than one week CPU time on a currently available PC. Fast equilibration of counterions was found to be important for observing frequent conformational transitions in the DNA oligomers. The averaged structures show the characteristics of B-form DNA with sequence-dependent helical step parameters that are close to the averages calculated for the ten different dinucleotide steps from crystallographic databases.

Intrinsic DNA bending (Remo Rohs)

DNA bending is an important structural feature for indirect readout in protein-DNA recognition. The binding of papillomavirus E2 transcription factors to their DNA binding sites is associated with DNA bending. The consensus E2 target is of the general form ACCGN4CGGT with a variable four-base-pair region. This target sequence provides an attractive model system to study the origins of sequence-specific DNA bending by means of molecular simulations. We applied our new all-atom Monte Carlo (MC) algorithm that combines effective sampling with fast conformational equilibration. The resulting MC ensembles resemble very well the corresponding high-resolution crystal structures. Both in the experimental structures and in the simulations, distinct bending is observed for the E2-DNA binding site with central AATT linker, in contrast to an essentially straight DNA with central ACGT linker. Structural contributions of specific base pair steps to the overall DNA bending have been analyzed in terms of helical parameters and local bend angles. The calculated contributions to the conformational energy, found for different states along the trajectory, have led to new insights into energetic origins that cause and stabilize intrinsic DNA bending.



The figure illustrates the binding-mode transition of Methylene Blue (MB) in the complex with DNA, as observed in a Monte Carlo (MC) simulation. Snapshots are shown throughout the transition from MB intercalation **(A)** to minor-groove binding **(D)** in two different orientations: a view into the central minor groove (upper panel) and a view perpendicular to that direction (lower panel). The two DNA strands are shown in green and red, the van der Waals surface of the DNA in gray and that of MB in blue.

Flexible drug-DNA docking (Remo Rohs)

The dynamics of biological processes depends on the structure and flexibility of interacting molecules. In particular, the conformational diversity of DNA allows for large deformations induced by its binding to protein and drug molecules. Drug-DNA interactions are of high pharmaceutical interest since the mode of action of anticancer, antiviral, antibacterial, and other drugs is directly associated with their binding to DNA. A reliable prediction of drug-DNA binding at the atomic level by molecular docking methods provides the basis for the design of new drug compounds. Using methylene blue (MB) as a model system and taking into account the flexibility of both the ligand and its DNA target, it has been demonstrated that our new algorithm results in an efficient MC docking of MB to DNA. The binding of MB at the minor groove emerged fast in the majority of the docking trials in accordance with experimental data. In addition to the preferred binding at the DNA minor groove, the docking trials resulted in a preference of MB binding to AT-rich over GC-rich base sequences, which is in agreement with spectroscopic data. Our results show that MC simulations of MB-DNA interactions sample both binding sites and binding modes, as MB intercalated between AT base pairs migrates into the minor groove, whereas the DNA deformation upon intercalation was reversed. Hence, the effect of the base sequence on drug-DNA binding modes becomes hereby a realistic object of molecular modeling.

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Nucleoside Analogs as Inhibitors of HBV and HCV Replication

Eckart Matthes

Chronic hepatitis B affects 350 million people worldwide and is responsible for 1 million deaths per year caused by cirrhosis and hepatocellular carcinoma. Both could be prevented by an effective antiviral therapy. Treatment with the L-nucleoside analog lamivudine (3TC), the first approved oral drug for chronic hepatitis B virus (HBV) infection, is highly effective. However, the prolonged treatment required for complete virus clearance leads to selection of drug resistant HBV strains compromising the efficacy of lamivudine.

Antiviral therapy with two and more agents might minimize the risk of resistance and produce a sustained reduction of virus load as demonstrated by the combination therapy against HIV infection. Based on this experience, combination therapy provides a promising approach also for an effective anti-HBV therapy. There is an urgent need for new antiviral drugs sufficient for combination therapy. Several new nucleosides with synergistic effects are at various stages of development.

We have synthesized new L-nucleosides and their corresponding triphosphates targeting the catalytic site of the C subdomain of HBV DNA polymerase. In cooperation with H. Will, Heinrich-Pette-Institut, Hamburg, we have studied the new compounds in HepG2 2.2.15 cells transfected with the HBV genome. Between the newly developed compounds, β-L-2', 3'-didehydro-2',3'-dideoxy-N4-hydroxycytidine (L-hydroxy-ddeC) proved to be one of the most active inhibitor of HBV replication ever detected ($EC_{50} < 0.05$ µM). In contrast to other extremely effective compounds, L-hydroxy-ddeC displays very low cytotoxicity in different proliferating cell lines ($CD_{50} > 1500$ µM). Other promising agents we developed are β-L-2', 3'-dideoxy-N4-hydroxycytidine (L-hydroxy-ddC) and N4-hydroxythiacytidine (hydroxy-3TC) with ED_{50} values of 0.35 and 0.7 µM, respectively, and CD_{50} values > 1000 µM.

3TC itself is also known as a strong inhibitor of HIV replication. Therefore, we assayed hydroxy-3TC for anti-HIV activity. Surprisingly, our new compound is completely inactive against HIV suggesting that this analog is not metabolized to 3TC. Elucidation of the metabolism of hydroxy-3TC and of the other mentioned β-L-hydroxycytosine nucleosides are under way. The triphosphates of the compounds were detected as strong inhibitors of HBV DNA polymerase supporting further the idea that the 4-NHOH-group itself is responsible for the efficiency of the agents. Lamivudine (3TC) resistance has been associated with substitution of isoleucine (I) or valine (V) for methionine in the YMDD motif at position 552 of HBV DNA polymerase, based on a molecular model of HBV polymerase derived from the structure of the homologous retroviral reverse transcriptases.

The YIDD containing HBV genome was constructed and transfected into C3A cells which produce a high level of HBV mutants. Preliminary results suggest that both 3TC and hydroxy-3TC are less effective. However, a quantitative estimation of the sensivity of this and other compounds against the HBV mutant remains to be obtained. In each ease, the replacement of the L-oxathiolane ring of hydroxy-3TC by sugar derivatives avoiding structures considered as mainly responsible for the resistance.

β-L-hydroxy-ddeC has exceptional potent activity against HBV replication which merits support for further development as potential anti HBV agent.

The development of an effective treatment of hepatitis C virus (HCV) infections represents a challenge of similar dimensions as for HBV infections. In contrast to HBV, there is no approved selective drug for treatment of HCV infections. In cooperation with R. Bartenschlager (Molecular Virology, Otto Meyerhof Center, University of Heidelberg), we designed and synthesized new ribonucleosides as potential inhibitors of RNA-dependent RNA polymerase (NS5B). Between a series of D- and L- ribonucleosides, a new compound, HYR2F, proved to be most effective in suppression of HCV replication in a Huh-7 cell-based replicon assay. Although a 90% inhibition of HCV replication was reached at 5 µM HYR2F, this concentration is also sufficient to inhibit cellular proliferation. Therefore, we focus our activity on modifications of this compound to increase its selectivity.

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Tumor Genetics

Siegfried Scherneck

The research program of the group is focused on projects aimed at understanding the genetic basis of human breast cancer (BC). BC is an extremely heterogeneous disease encompassing several pathological subtypes and a range of clinical manifestations. These are underpinned at the molecular level by a complex network of genetic alterations that affect cellular processes of tumor cells but also host cells that interact with the tumor, such as immune, vascular, and stromal cells.

The majority of breast cancer cases are so-called sporadic cancers that result from the accumulation of acquired and uncorrected genetic alterations in somatic cells. At present, our knowledge about sporadic BC-associated genes, their functions and interactions in pathways regulating growth and arrest of cells, is limited. New technological developments offer powerful means to analyze the activity of thousands of genes at the DNA, RNA, and protein level. They have proven to be valuable in identifying genes and pathways associated with different subtypes of BC, response to treatment, and prognosis.

About 5-10% of BC are thought to be due to highly penetrant inherited predisposing mutations in at least two genes, designated as BRCA1 and BRCA2. It is expected that at least one more susceptibility gene will emerge. Multiple approaches, such as linkage analysis in high-risk families and association studies in large BC case-control cohorts, provide the basis to identify additional high- but also low-penetrance genes.

Identifying and managing hereditary risk for breast cancer – implications for medical care

W. Hofmann, V. Gimmel, I. Berger, A. Pietschmann, M. Hinzmann, H. Zeidler in cooperation with 11 centers of the "German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC)" and the "Interdisciplinary Breast Center", Charité, Berlin.

The BRCA1 and BRCA2 genes account for autosomal dominant transmission of susceptibility in a majority of families with hereditary breast and ovarian cancers. Women with a BRCA1/2 mutation have a lifetime BC risk of 50-85%. Within a nationwide interdisciplinary joint research project, supported by the "Deutsche Krebshilfe", we collected around 400 BC families for BRCA1/2 mutation analysis. We found and characterized more than 40 different mutations, which provide individual information about BC risk. A consensus-driven common structure was established by all members of the GCHBOC, and protocols for genetic counseling, risk estimation, and prevention were outlined to offer individuals of increased BC risk options for prophylactic surgery, chemoprevention, and increased surveillance. On the basis of these activities, the Health Insurance Companies in Germany began covering the costs for genetic testing for people with a family history of BC in January 2005.

Search for genes and gene networks involved in breast cancer

S. Seitz, B. Jandrig, C. Zeller, K. Wenzel, A. Schwartz, J. Strissel, R. Frege, M. Blankenburg, K. Michaelis, S. Werner, K. Daskalow, D. Gustavus in cooperation with I. Petersen (Charité, Berlin), N. Arnold (Kiel), A. Meindl (München), D. Niederacher (Düsseldorf), R. Schmutzler (Bonn), P.M. Schlag (RRK, Berlin), W. Arnold (atugen, Berlin), A. Rosenthal, B. Hinzmann (Signature diagnostics, Potsdam).

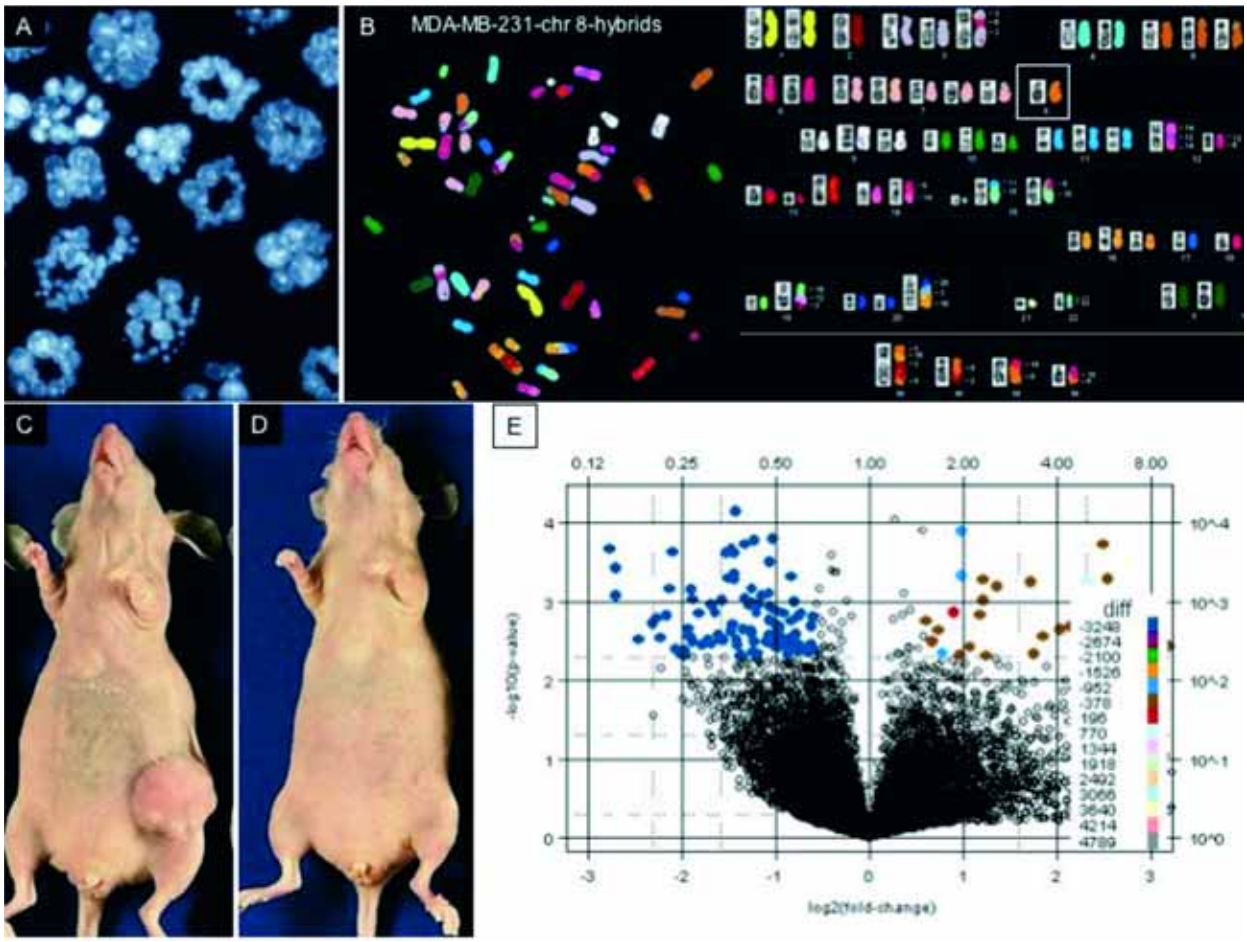
Over the last few years, a central aim of our research has been to identify genes whose tumor suppressor function is impaired or lost during BC development, with particular emphasis on chromosomes 6, 8, and 17. To identify and validate BC-associated genes, several positional and functional approaches are being used in combination.

Recently, we have developed a combined functional approach of microcell mediated chromosome transfer and expression difference analysis. This approach has identified a network of clinically and functionally relevant genes in a model of chromosome 6-, 8- or 17-mediated BC tumor suppression. The detailed contextual characterization of genes identified so far and other candidate genes and gene networks will determine the extent of their involvement in BC development.

Suppression of tumorigenicity in breast cancer cells by the microfilament protein profilin 1

B. Jandrig, I. Lapidous, A. Schwartz, K. Rücker, in cooperation with B.M. Jockusch (Braunschweig), P.M. Schlag (RRK, Berlin-Buch), W. Arnold (atugen, Berlin-Buch).

Profilin 1 (PFN1) is a regulator of the microfilament system and is involved in various signaling pathways. Our data show



A network of clinically and functionally relevant genes is involved in the reversion of the tumorigenic phenotype of MDA-MB-231 breast cancer cells after microcell mediated transfer of human chromosome 8.

A Microcells obtained after treatment of A9neo8 donor cells with colcemid for 48 h at 37°C

B Detection of an intact chromosome 8 in MDA-MB-231 hybrid cells by spectral karyotyping (SKY)

C MDA-MB-231 cells form tumors in nude mice

D MDA-MB-231 chr8 hybrid cells lost the ability to form tumors in nude mice

E Expression analysis identified a set of genes differentially expressed in MDA-MB-231 chr8 hybrid cells as compared to parental MDA-MB-231 cells

that human breast cancer cell lines as well as breast tumors express conspicuously low profilin 1 levels and adapt a non-tumorigenic phenotype upon raising their profilin 1 level. In an attempt to unravel the particular contribution of binding to different ligands in the tumor suppressor activity of profilin 1, we could conclude that the actin binding site on profilin 1 is instrumental for normal differentiation of human epithelia and the tumor suppressor function of profilin 1.

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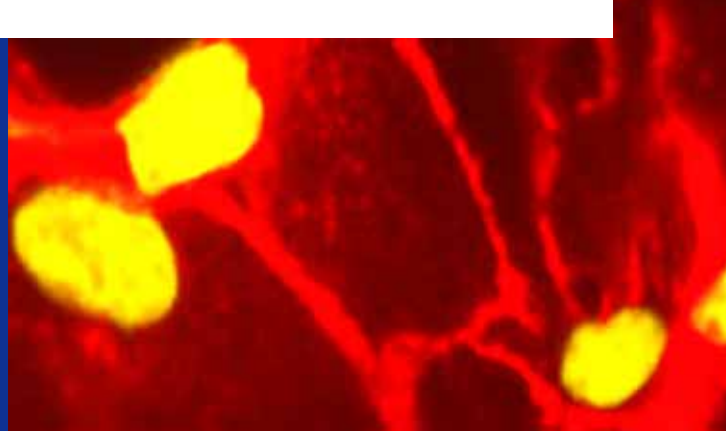


Differentiation and Growth Control in Lymphocyte Development and Immunopathogenesis

Martin Lipp

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 herpes viruses and the function of sphingophospholipid receptors in the immune system.

CXCR5-dependent antigen-specific lymphoid neo-organogenesis in a chronic model of rheumatoid arthritis

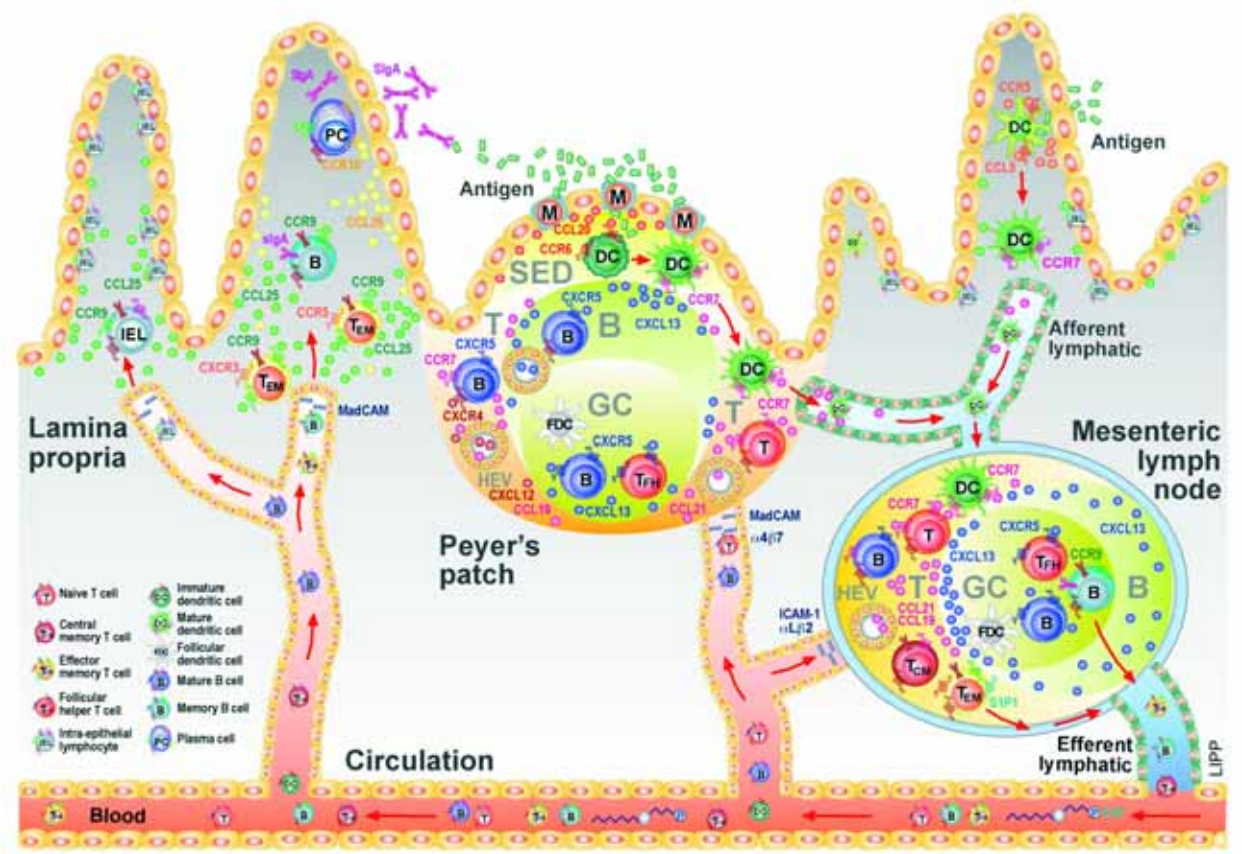
Rheumatoid arthritis (RA) is an inflammatory autoimmune disease with unknown etiology and only partially defined pathogenesis. We established a novel chronic mouse model of antigen-induced arthritis (AIA), in which development of ectopic lymphoid follicles are efficiently induced within the synovial tissue, a hallmark of human RA. Remarkably, all follicles showed topologically segregated B and CD4- and CD8-positive T cell areas and the formation of active germinal centers with proliferating lymphocytes. Importantly, for the first time, we showed that antigen-specific CD138+ plasma cells are generated in ectopic follicles and circulating autoantibodies directed against peptide C1 of collagen II are produced, which is also the most immunodominant collagen epitope in human RA. The development and organization of these ectopic structures were severely impaired in CXCR5- and CCR7-deficient mice proving that both chemokine receptors are critical signaling molecules in lymphoid neo-organogenesis 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 selfantigen-dependent interaction of memory/effector B and T lymphocytes resulting in aberrant chronic autoreactive immune responses.

CCR7 controls lymphocyte recirculation through non-lymphoid tissues and cellular homeostasis of the gastric mucosa

Homeostatic trafficking of lymphocytes through extralymphoid tissues is required for immune surveillance and the establishment of self-tolerance. However, the mechanisms regulating lymphocyte recirculation through peripheral tissues under non-inflammatory conditions are currently not well understood. We have been able to demonstrate that the chemokine receptor CCR7 controls not only lymphocyte trafficking to and within secondary lymphoid organs but also homeostatic recirculation of lymphocytes through body cavities and non-lymphoid tissues. $CCR7^{-/-}$ mice show lymphocyte accumulation in body cavities due to impaired lymphocyte egress. The gastrointestinal mucosal tissue of $CCR7^{-/-}$ mice is highly permissive for the formation of ectopic lymphoid follicular structures. Moreover, CCR7 deficiency induces age-dependent histomorphological changes in the stomach with profound cystic hyperplasia and mucosal proliferation resembling Menetrier's disease. Thus, CCR7 regulates the cellular organization of visceral tissue by governing recirculation of naive and memory lymphocytes under homeostatic conditions.

CXCR5 and CCR7 regulate peritoneal B cell homing and early immunity against bacterial pathogens

CXCR5 and CCR7 are thought to regulate homing and microenvironmental localization of B lymphocytes during antigen-independent and -dependent B cell differentiation. To assess the roles of CXCR5 and CCR7 in early immune responses, we examined B lymphocyte subpopulations in the peritoneum of mice lacking one or both chemokine receptors. Mice lacking CXCR5 showed a severe paucity of peritoneal B-1 cells and conventional B-2 cells. CCR7 deficiency resulted in a partial reduction in B-1 cells and, most strikingly, in a massive increase in B-2 cells. Antigen-induced phosphorylcholine-



Chemokine-encoded address codes for lymphoid cell migration in mucosal immunity

In terms of immune functions the tissue of the small intestine can be divided into the initiation compartment defined as gut-associated lymphoid tissue (GALT), which includes the organized structures of Peyer's patches (PP) and mesenteric lymph nodes (MLN), and into the effector compartment consisting of the lamina propria (LP) with scattered effector T and B cells, and of the intraepithelial lymphocytes (IEL) embedded in the epithelial cell layer of the intestinal wall. The cell type and tissue-specific expression of chemokines and their corresponding receptors regulates lymphocyte and dendritic (DC) cell homing to mucosal tissues.

Naïve, recirculating T cells leave the bloodstream in high endothelial venules, (HEVs) crossing the T cell rich area of PPs, which depends on expression of the chemokine receptor CCR7 (which binds CCL19 and CCL21). In contrast, naïve B cells predominantly employ CXCR5 (which binds CXCL13) to leave the bloodstream in follicular HEVs. B cells may also utilize CXCR4 or CCR7 to enter PPs; however, both receptors cannot compensate for a lack of CXCR5. Moreover, CCR7 and CXCR5 are responsible for the organization of secondary lymphoid tissue into B cell-rich and T cell-rich zones.

DCs depend on CCR6 to enter the sub-epithelial dome (SED) region of PPs. CCL20, the ligand for CCR6 directs CD11c+CD11b+ DCs in close proximity to M cells, that actively acquire antigen from the intestinal lumen. Following antigen uptake, maturating DCs upregulate CCR7, which enables these cells to migrate via the afferent lymphatic vessels into the interfollicular T cell rich regions of PPs or the T cell zones of MLN.

T cell activation by DCs confers to T cells the ability to home to non-lymphoid organs. Homing of central memory/effector memory T cells to the LP of the small intestine involves CCR9. The ligand for CCR9, CCL25, is constitutively expressed by epithelial cells of the lower villi and crypts within the small intestine and deposited on the luminal surface of epithelial cells in intestinal venules. In addition, expression of receptors for inflammatory chemokines, such as CXCR3 and CCR5, contribute to the homing of effector T cells (TEM) to the LP.

IgA-secreting plasma cells (PC), which are generated in germinal centers (GC) of PP and MLN, utilize CCR9 to home to the LP of the small intestine. Once PC have entered the mucosal effector tissue, CCR9 becomes downregulated. Most IgA-secreting PC start to express CCR10 and show chemotactic response towards CCL28, the ligand for CCR10 that is constitutively expressed by epithelial cells in the small and large intestine. A subset of circulating memory surface IgA$^+$ B cells developed in GC of PP and MLN expresses CCR9. After activation in lymphoid organs, lymphocytes must again return to circulation to reach effector sites. Upregulation of the sphingosine-1-phosphate receptor S1P1 in memory/effector T and B cells in course of ongoing immune responses enables these cells to egress from secondary lymphatic organs and enter the circulation. *(M. Lipp in Cell Migration in Development and Disease, D. Wedlich (ed.), Wiley-VCH, Weinheim, 2004)*

specific IgM responses after intraperitoneal administration of streptococcal antigen were reduced in all three knockout strains. In addition, CCR7$^{-/-}$ mice had enhanced splenic IgM$^+$ plasma cell responses. Thus, the two chemokine receptors exert divergent forces at multiple levels of the innate immune response. CXCR5 plays a dominant role in peritoneal B-1 B cell homing and body cavity immunity, but both chemokine receptors are needed for a proportional peritoneal B-2 cell homing and balanced development of an early splenic B cell response.

Critical role of CCR7 in cytotoxic T cell priming in alloimmune responses and during Listeria monocytogenes infection

(in cooperation with J. Droese; H.-G. Zerwes, Novartis, Basel)

The requirement of CCR7, which regulate co-localization of T cells and mature dendritic cells within secondary lymphoid organs, in efficient priming of allo-specific cytotoxic CD8$^+$ T-cells is poorly characterized. We could demonstrate a critical role for CCR7 in the initiation of an alloimmune response and in the development of transplant rejection. Remarkably, in

a model of acute allogeneic tumor rejection, CCR7$^{-/-}$ mice completely failed to reject subcutaneously injected MHC class I mismatched tumor cells and cytotoxic activity of allospecific T cells was severely compromised. When solid tumors derived from wild type mice were transplanted, recipient CCR7$^{-/-}$ mice were capable of rejecting the allografts. In contrast, tumor allografts transplanted from CCR7$^{-/-}$ donors onto CCR7$^{-/-}$ recipients showed allograft survival up to 28 days, suggesting a critical function of CCR7 on donor-type passenger leukocytes in the initiation of cytotoxic CD8$^+$ T cell responses. In a heterotopic heart transplantation model CCR7 deficiency resulted in significantly prolonged but not indefinite allograft survival. Our results define a key role for CCR7 in allogeneic T cell priming within the context of draining lymph nodes.

(in cooperation with M. Kursar, H.-W. Mittrücker and S. H. E. Kaufmann, MPI for Infectious Biology, Berlin)
In line with the described function of CCR7 in allospecific cytotoxic immune responses, we found that the activation of naïve MHC class Ia-restricted CD8$^+$ T cells after *L. monocytogenes* infection markedly depends on CCR7. CCR7 has to be expressed on both CD8$^+$ T cells and professional antigen presenting cells to allow efficient MHC class Ia-restricted priming. In contrast, MHC class Ib-restricted CD8$^+$ T cells and MHC class II-restricted CD4$^+$ T cells showed only some dependency and activation of MHC class Ia-restricted CD8$^+$ memory T cells was virtually independent of CCR7. The partial requirement of the T cell response is also reflected by the relative resistance of CCR7$^{-/-}$ mice during primary and secondary *L. monocytogenes*-infection.

Expression of CCR7 and CXCR5 Defines Functionally Distinct T Cell Subsets

Within peripheral blood, expression of CCR7, CXCR5, and CD62L allow for the identification three functionally distinct subsets of memory/effector CD4$^+$T cells. CCR7-CD62L-effector memory T (T_{EM}) cells have downregulated CCR7 and most closely resemble classical effector cells. In contrast, expression levels of CCR7 and CD62L remain high on a second subset of memory/effector cells. These cells appear to retain the capacity to home to secondary lymphoid organs and have therefore been named central memory T (TCM) cells. A third population, about 15 % of human peripheral blood CD4$^+$ T cells, expresses CXCR5 along with CCR7, and was provisionally named TCM1. Within secondary lymphoid organs, the population of CXCR5$^+$CD4$^+$ T cells is significantly enlarged. The upregulation of CXCR5 is accompanied by a downregulation of CCR7 and consequently these cells are able to enter B cell follicles. CD4$^+$CXCR5$^+$ T cells located within germinal centers express costimulatory molecules such as ICOS and act as B helper T cells in that they promote the antibody secretion by B cells. For this reason, we have named these cells follicular T helper B (T_{FH}) cells. However, the origin and fate of CXCR5$^+$CD4$^+$ T cells is still under discussion. Currently, we are analyzing these CD4$^+$ memory/effector T cell populations in order to better understand their differentiation pathway and their role in chronic inflammatory and infectious diseases.

Immune modulatory and growth-inducing functions of viral chemokine receptors: A murine model for Kaposi's sarcoma

(in cooperation with I. Anagnostopoulos, H. Stein, FU; K. Kölble, Charité)

Infection with HHV-8 has been linked by epidemiological and molecular evidence to the pathogenesis of all forms of Kaposi's sarcoma, a non-Hodgkin's B cell lymphoma, and multicentric Castleman's disease (MCD). The research project is aimed to establish whether the HHV-8-encoded chemokine receptor (vGPCR) plays a critical role in the development of HHV8-associated diseases and malignancies as an essential oncogenic and paracrine factor. We have used retroviral transduction to generate vGPCR-expressing NIH3T3 cell lines 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 immunocompentent BALB/c mice. Unexpectedly, vGPCR expressing cells established from grafted tumor fragments give rise to angioproliferative fibrosarcomas in immunocompetent mice, which exhibit a striking histological resemblance to KS including spindle cell morphology, a high degree of vascularization and brisk mitotic activity. High expression of the vGPCR was confirmed in both the cell lines and tumors by vGPCR-specific staining. This novel animal model of KS might contribute to the understanding of the underlying molecular mechanisms promoting vGPCR-associated oncogenesis and immune evasion, and will facilitate the development of vGPCR-specific vaccination strategies.

Role of sphingophospholipid receptors in the immune system

Sphingosin-1-phosphate (S1P) receptors are a group of G protein coupled receptors mediating a wide variety of biological responses. Our laboratory has initially described the $S1P_4$ receptors. This receptor with an as yet unidentified function is highly expressed on cells of the hematopoietic and lymphoid system. A second receptor highly expressed in those tissues, $S1P_1$, has recently been shown to regulate the egress of T cells from secondary lymphatic organs. In order to differentially assess the effects of $S1P_1$ and $S1P_4$ stimulation on the homeostasis of the immune system and the generation of an immune response, we have generated S1P4$^{-/-}$ mice as well as lentiviral vectors for siRNA-mediated gene knock-down of the $S1P_1$ gene in lymphocytes. These models will be used for the investigation of the role of the $S1P_4$ receptor in the immune homeostasis.

Mechanisms of lymphatic metastasis of solid tumors with special focus on lysophospholipid- and chemokine receptors

(in cooperation with P. M. Schlag, Charité)

Lymphatic metastasis at diagnosis is present in up to 25% of patients suffering from gastric cancer and is in the majority of cases responsible for the fatal outcome of the disease. The molecular mechanisms leading to metastasis appear to occur early in tumor development. We used laser capture micro-

dissection to obtain highly purified cell populations from node-negative and node-positive primary gastric tumors as well as from the corresponding normal gastric mucosa and subsequently performed gene expression profiling to identify candidate genes implicated in the development of lymph node metastasis. Non-supervised clustering of expression profiles from tumor tissue revealed clearly distinguishable profiles from tumors with and without lymphatic metastasis. In contrast, expression profiles from normal gastric mucosa of patients with and without lymph node metastases could not be differentiated. By comparison of expression profiles from metastatic and non-metastatic primary tumors, we were able to define a significant number of genes at least 3 fold up- or downregulated in metastasised gastric cancer. The detailed biostatistical analysis is currently ongoing.

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Biology and Targeted Therapy of Lymphoma

Bernd Dörken

Scope

Studying the molecular mechanisms underlying B cell development and differentiation is one of the key approaches to understanding the pathways leading to disease. We are particularly interested in terminally differentiated B cells that give rise to two hematologic malignancies, Hodgkin´s lymphoma and multiple myeloma. Hodgkin and Reed-Sternberg cells are tumor cells of classical Hodgkin's lymphoma (cHL). In most cases, they are derived from germinal center B cells. However, they do not express immunoglobulins and typical B-cell markers on their cell surface. We focus our work on the characterization of the molecular basis for the dedifferentiated B cell phenotype of Hodgkin's lymphoma and aim to identify molecular defects that are responsible for tumor cell transformation and differentiation. We further evaluate molecular targets in multiple myeloma where tight interactions between neoplastic myeloma cells and their microenvironment drive tumor cell proliferation. Inhibition of the deregulated Notch system in multiple myeloma is currently investigated as a novel treatment option.

Characterization of deregulated transcription factor networks in Hodgkin´s lymphoma

S. Mathas, M. Janz, A. Lietz in cooperation with C. Scheidereit (MDC) and H. Stein (Charité)

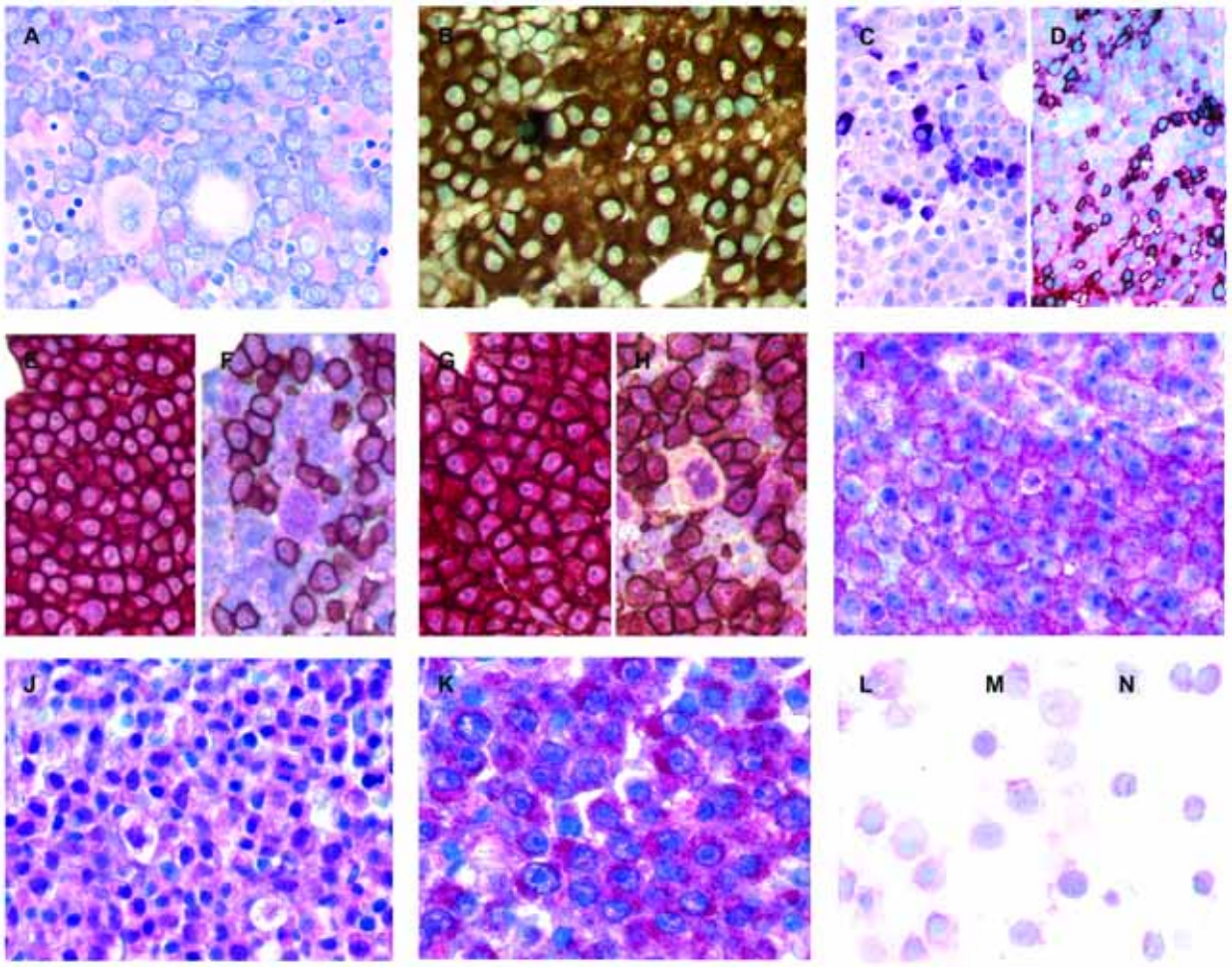
Using classical Hodgkin's lymphoma (cHL) as a model system, we are investigating the role of transcription factors in lymphoma development. Malignant transformation of hematopoietic cells is associated with profound alterations in the transcriptional program resulting in deregulated proliferation, differentiation, and apoptosis. Using oligonucleotide microarrays, we have generated expression profiles for cHL-derived cell lines as well as Non-Hodgkin B-cell lines. These microarray data provide the basis for the identification of differentially expressed genes and transcription factor networks that determine the malignant phenotype of cHL. In detail, we established that cHL the B cell determinating transcription factor network, mainly consisting of the transcription factors E2A, EBF, and Pax5, is functionally disrupted in HRS cells. Regarding the underlying pathomechanism, we identified overexpression of the helix-loop-helix proteins activated B cell factor 1 (ABF-1) and inhibitor of differentiation 2 (Id-2). Both proteins show a strong, aberrant expression in HL cell lines and Hodgkin and Reed-Sternberg cells of primary HL cases. Importantly, these factors are able to down-regulate B cell-specific genes and to allow up-regulation of B-lineage inappropriate genes. Together, the altered expression of these factors offers an explanation for the unique HL phenotype. In continuation of earlier work, we focus on the analysis of the NF-κB and AP-1 transcription factor systems not only in HL but also in related lymphoma types such as anaplastic large cell lymphoma (ALCL). Recent results show that overexpression of the NF-κB/IκB family member Bcl-3 reveals a novel molecular defect of the NF-κB system in cHL and ALCL. Identification of such key regulatory gene defects provides a rational basis for the development of novel targeted therapeutics for lymphoma therapy.

Targeted therapy of Hodgkin´s lymphoma and multiple myeloma

F. Jundt, R. Schwarzer, Ö. Acikgöz, N. Rätzel, U. Ellinghaus in cooperation with H. Stein (Charité)

Hodgkin's lymphoma (HL) and anaplastic large cell lymphoma (ALCL) share morphologic and immunophenotypic markers in a subgroup of cases although they are biologically distinct entities. Moreover, in both entities, novel therapeutic options are needed as curative therapy of HL is compromised by a high risk of long-term complications and anaplastic lymphoma kinase (ALK) negative ALCL still have a very unfavorable prognosis with current treatment strategies. The macrocyclic lactone SDZ RAD (RAD, INN: everolimus) is a rapamycin derivative with potent immunosuppressive and anti-proliferative properties. Here, we investigated whether RAD inhibits tumor cell proliferation of HL and ALCL. We show that RAD strongly inhibits proliferation of HL and ALCL cells *in vitro* and in NOD/SCID mice *in vivo*. Moreover, we identified two molecular mechanisms that show how RAD exerts anti-proliferative effects in HL and ALCL cells. RAD down-regulates the truncated isoform of the transcription factor CCAAT enhancer binding protein (C/EBP)α that is known to disrupt terminal differentiation and induce a transformed phenotype. Furthermore, RAD inhibits constitutive NF-κB activity, i.e. interferes with a critical survival factor of HL cells. Pharmacological inhibition of the mTOR pathway by RAD therefore targets an essential proliferation and survival pathways in HL and ALCL cells and might serve as a novel treatment option.

Another therapeutic opportunity comes from analysis of deregulated Notch signaling. Notch1 belongs to a family of transmembrane receptors that control cell proliferation and differentiation in response to extracellular ligands expressed on neighbouring cells. These receptors are expressed on hematopoietic stem cells and interact with their ligands on bone marrow stromal cells and, thereby, control cell fate decisions and survival. We investigated whether Notch signaling is involved in the tight interactions between neoplastic plasma cells and



High Notch1, Notch2 and Jagged1 expression in primary MM cells. (A-H) Bone marrow biopsy specimen of one case of MM. (A) Giemsa staining demonstrating bone marrow infiltration by atypical plasma cells (MM cells) with large nuclei and prominent nucleoli. (B) MM cells show monotypic expression of immunoglobulin light chain kappa. Immunostains for myeloperoxidase (C) and glycophorin C (D) mark granulocytic and erythroid precursors showing that MM cells are surrounded by non-malignant bone marrow cells. (E, F) Double labeling for CD138 (brown reaction product; streptavidin/biotin/peroxidase technique) and Notch1 (red reaction product; immunoalkaline-phosphatase method) reveals that CD138 positive MM cells strongly co-express Notch1. (G, H) Double labeling for CD138 (brown reaction product) and the Notch ligand Jagged1 (red reaction product) demonstrates co-expression of CD138 and Jagged1 on MM cells. (F, H) Some CD138 negative non-malignant bone marrow cells, mainly megacaryocytes, show weak immunoreactivity against Notch1 or Jagged1. (I-K) Extramedullar (liver) biopsy specimen of MM immunostained for Notch1 (I), Notch2 (J) and Jagged1 (K) and counterstained with hematoxylin. MM cells are intensely labeled by anti-Notch1, anti-Notch2 and anti-Jagged1 antibodies. (L-N). Isolated plasma cells ($CD38^{+++}/CD19^{+}$) of non-neoplastic bone marrow of normal donors (cytospins) show low to undetectable immunoreactivity against Notch1 (L), Notch2 (M) and Jagged1 (N). Original magnification x 200.

their bone marrow microenvironment which are essential for tumor cell growth in multiple myeloma (MM). Notch receptors and their ligand Jagged1 are highly expressed in cultured and primary MM cells, whereas their non-neoplastic counterparts show low to undetectable levels of Notch. Functional analyses indicate that ligand-induced Notch signaling is a growth factor for MM cells and suggest that these interactions contribute to lymphomagenesis of MM *in vivo*. Therapeutic manipulation of Notch signaling is currently explored.

Non-genotoxic activation of the p53 pathway as a therapeutic strategy in multiple myeloma

R. Bargou, K. Bommert, T. Stühmer, M. Chatterjee, S. Lentzsch, P. T. Daniel in cooperation with L. T. Vassilev (Discovery Oncology, Hoffmann-La Roche Inc., Nutley, NJ, USA)

Mutation of p53 is a rare event in multiple myeloma but it is unknown if p53 signaling is functional in myeloma cells and if targeted non-genotoxic activation of the p53 pathway is sufficient to kill tumor cells. We have demonstrated that treatment of primary tumor samples with a small-molecule inhibitor of the p53/murine double minute 2 (MDM2) interaction increases the level of p53 and induces p53 targets and apoptotic cell death. Significantly, given the importance of the bone marrow microenvironment for the support and drug resistance of myeloma cells, tumor cells undergo effective apoptosis also in the presence of stromal cells which themselves appear to tolerate exposure to nutlin-3. The *in vitro* toxicity of nutlin-3 was similar to that of the genotoxic drug melphalan. Since nutlin-mediated p53 activation is not dependent on DNA damage, MDM2 antagonists may help to avoid or reduce the severe genotoxic side effects of chemotherapeutic agents currently used to treat multiple myeloma. Therefore, MDM2 antagonists may offer a new treatment option for this disease.

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Molecular Mechanisms of Immune Evasion in Tumor Biology and Herpesvirus Infection

Armin Rehm
(Helmholtz Fellow)

Scope

Our work is focused on the role of immunosurveillance in tumor biology and in Herpes virus infections. Tumor cells employ a multitude of mechanisms to evade the hosts immune response, and some of those mechanisms can be linked conceptually to the strategies persistent Herpesviruses employ for their own benefit. The molecular and systemic study of those immunoevasive strategies is a prerequisite for improving current immune system-based treatment options.

Physiological and pathophysiological role of the tumor-associated antigen EBAG9

(in cooperation with U.E. Höpken, R. Jüttner, F. Rathjen, C. Birchmeier-Kohler, B. Erdmann, W. Uckert, P. Daniel)

In tumor immunology, considerable effort has been taken to discover tumor-associated antigens. In addition to being diagnostic markers, they also serve as targets in the development of tumor-specific immunotherapies. Recently, a novel tumor-associated antigen that was attributed a function in tumor immune escape, EBAG9, was described which causes the deposition of the O-linked glycans (Tn and TF) on non-secretory cell lines. The identification of the SNARE-associated molecule Snapin as an interaction partner pointed to an involvement of EBAG9 in the control of the regulated secretory pathway. We have applied a multidisciplinary approach to study the pathways and functional consequences of EBAG9-related O-linked glycan expression, among them its role in tumor cell adhesion, invasiveness, and, ultimately, metastasis. We plan to test the hypothesis as to whether EBAG9 has a more general role in the regulation of diverse vesicle trafficking routes and determine if this function can provide a link to tumor pathogenesis. *In vivo*, we study the consequences of a genetic deletion of EBAG9 in a knockout mouse model. Here, we focus on the analysis of the secretory function in endocrine, immune, and neuronal cells.

Expression and functional significance of immunomodulatory molecules in primary-mediastinal large B cell lymphoma (PMBL) and thymic B cells

(in cooperation with U.E. Höpken, M. Lipp; M. Brömer, C. Scheidereit; C. Schmitt; MDC; I. Anagnostopoulos, H. Stein, Charité; M. Hübler, Deutsches Herzzentrum)

A hallmark of tumor cell malignancy is its intrinsic capacity to leave the primary site of origin and to undergo migration and invasion. The underlying mechanisms recapitulate those that are effective in non-neoplastic cells, among them lymphocyte trafficking. Since these processes are orchestrated by the chemokine system, we focus in this project on the analysis of the ligand and receptor profile in primary-mediastinal large B cell lymphoma. We have elucidated a common trait between mediastinal B cell lymphoma cells and their putative ancestors, thymic B cells, but we found also markers that separate both populations. Based on the published gene array analysis of PMBL, we focused on several genes that have a putative immunomodulatory function. Those genes will be studied functionally in transgenic mouse models to score for their impact on lymphoma progression in an immunocompetent environment.

Role of the HCMV encoded chemokine receptor US28 in immune evasion

(in cooperation with U.E. Höpken, M. Lipp)

The HCMV encoded chemokine receptor, US28, serves as a decoy for inflammatory chemokines. A pathophysiological relevance of this receptor has been suggested for HIV-infections and atherosclerosis as well as for the reduction of immune responses during reactivation of HCMV infections. Since surface deposition of the receptor is usually very low, we studied the mechanisms of its endocytosis. Although an US28-dependent redistribution of β-arrestin into endosomes occurred, endocytosis of US28 was independent of β-arrestin. Instead, internalization was dynamin-dependent. Whereas the clathrin-coated pit pathway was predominantly employed, cholesterol depletion, which is indicative of a lipid raft or caveolae pathway, was also effective. The simultaneous employment of diverse endocytosis routes identifies US28 as a chemokine receptor homologue with an unusual flexibility.

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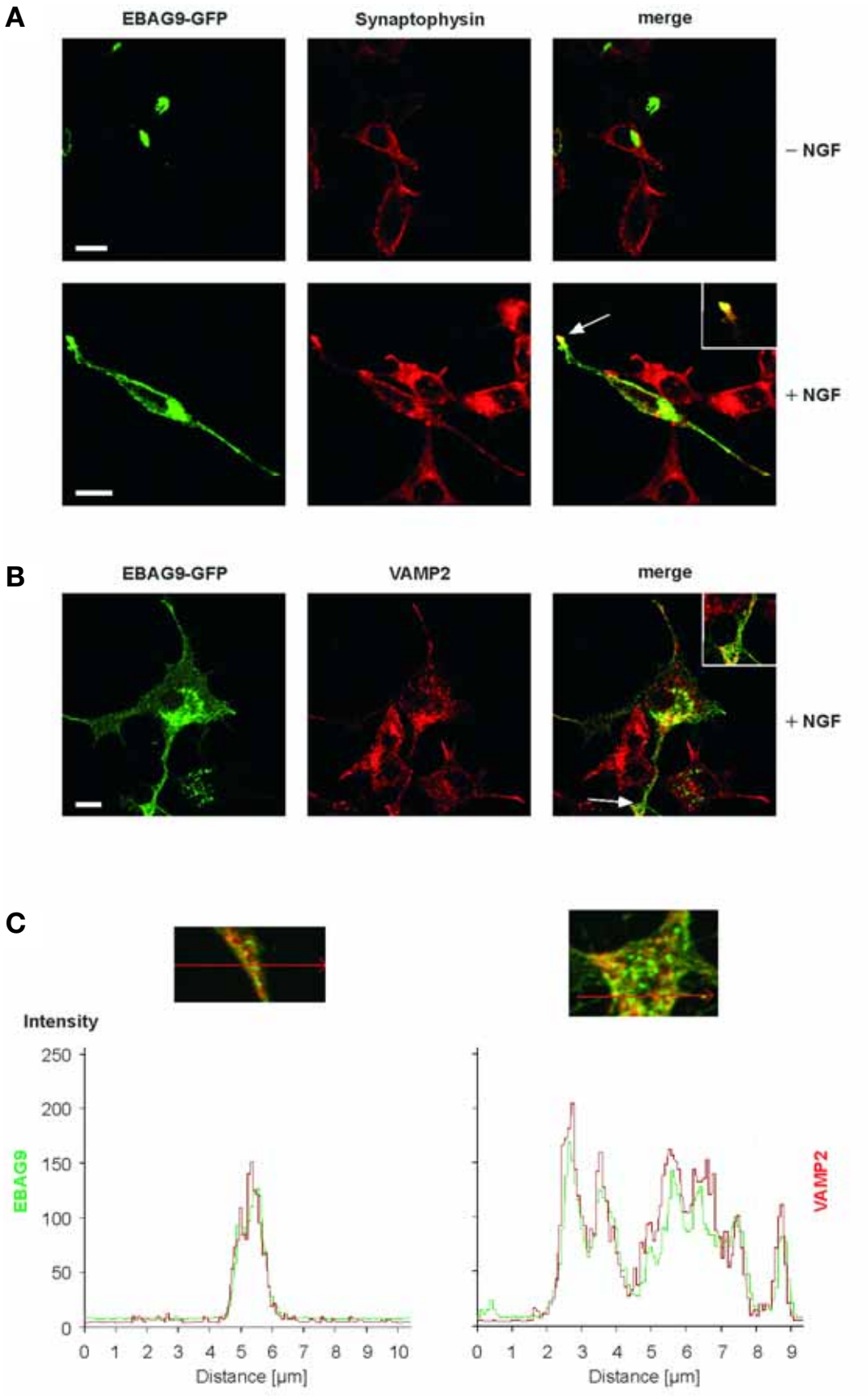

EBAG9 is subject to a dynamic redistribution in differentiated neuroendocrine cells.
PC12 cells were transfected with EBAG9-GFP (green) and cultured in the absence (-) and presence (+) of NGF. Cells were stained with antibodies against synaptic vesicle markers, Synaptophysin (A, red) and VAMP2 (B, red), respectively, and analyzed by confocal microscopy. Merged images are shown on the right. (C) Magnification of overlaid EBAG9-GFP and VAMP2 in neurite extensions and in the cell soma. The fluorescence intensities measured along a line were plotted as numbers of pixels (y-axis) relative to their position along the region (x-axis).

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Cell-Biological Determinants of Treatment Response and Prognosis in Acute Leukemias

Wolf-Dieter Ludwig

Scope

Recent and ongoing projects of the Acute Leukemia Research Group focus on identification of cellular and molecular factors mediating drug resistance in normal and malignant hematopoietic cells as well as their prognostic impact in acute leukemias. These studies have been embedded in the framework of international multicentre trials in childhood (ALL-BFM) and adult (AMLCG) acute leukemias, thereby providing the opportunity to investigate biologically and clinically well-characterized leukemic specimens and to correlate biological findings with treatment response and prognosis.

Mitochondrial activity is a critical determinant of stress-induced activation of the p53 tumor suppressor in leukemia cells and normal lymphocytes

The p53 system is highly stress-sensitive and integrates diverse intracellular signals in a complex and poorly defined manner. Mitochondrial activity is one of the critical intracellular determinants involved in the energy metabolism and cellular redox state. Mitochondrial involvement in pro-apoptotic p53 signaling has been largely investigated downstream of p53 activation in cellular systems over-expressing either wild-type (wt) or temperature-sensitive mutant p53. Using cellular systems with endogenously expressed wt p53, including normal T lymphocytes and leukemia blasts, we addressed the contribution of mitochondrial components to the early stress signaling upstream of p53 activation. Down-regulation of mitochondrial activity and mitochondrial transmembrane potential by the inhibitor of ATP synthesis (oligomycin) selectively abrogated p53-dependent but not p53-independent apoptosis. Oligomycin prevented stress-induced p53 protein accumulation and up-regulation of the p53 transcriptional targets. Oligomycin caused only a slight reduction of intracellular ATP levels but specifically decreased levels of reactive oxygen species (ROS) localized to mitochondria. Given that stress-induced p53 activation showed strong ROS sensitivity, these observations identified mitochondrial activity, described as mitochondrial transmembrane potential and ROS levels, as a critical intracellular determinant of the p53 stress sensitivity.

Cytochrome c-related caspase-3 activation determines treatment response and relapse in childhood ALL

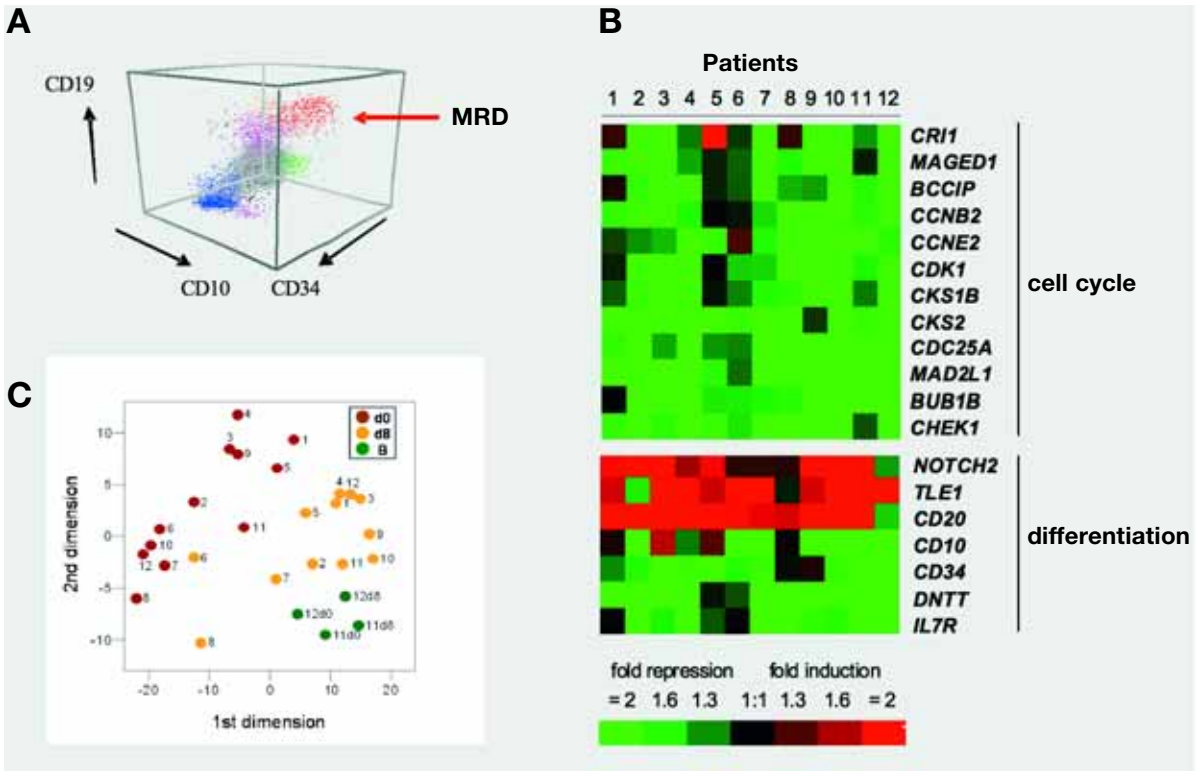
(in cooperation with K. Stahnke and K.-M. Debatin, University of Ulm)

Deficient activation of apoptosis signaling pathways may be responsible for treatment failure in acute leukemia. A hallmark of the drug-induced mitochondrial apoptosis signaling is the mitochondrial release of apoptogenic factors, in particular of cytochrome *c*, which in turn mediates activation of the effector caspase-3. To investigate these key events in primary leukemia cells, we developed a flow-cytometric method for detection of mitochondrial cytochrome c release and caspase-3 activation by conformation-sensitive antibodies. Using this method, we investigated cytochrome c-related caspase activation in patients with pediatric precursor B-cell ALL (PBC-ALL). Both events correlated only in the group of good responding patients and patients in continuous remission. By combining both parameters, we have identified a novel indicator (cytochrome c related activation of capase-3, CRAC) which directly connects the extent of caspase-3 activation to cytochrome c release in single cells in an individual patient sample. In the investigated series of ALLs, patients with positive CRAC values revealed a significantly higher probability of relapse-free survival than CRAC-negative patients. These data indicate that constitutive differences in the activation of post-mitochondrial apoptosis signaling pathways significantly determine therapy response in childhood ALL.

Monitoring and characterization of minimal residual disease (MRD) cells in acute leukemia

(in part within the framework of the CancerNet Berlin/National Genome Research Network)

Recent studies in acute leukemias have demonstrated that monitoring of MRD by flow-cytometric immunophenotyping and PCR techniques is useful for evaluating early response to treatment and predicts clinical outcome. We, therefore, investigated prospectively MRD detection by multi-parametric flow cytometry and its impact in the disease monitoring in ALL. A comprehensive panel of antigen markers has been established to reliably identify the leukemia-associated immunophenotype (LAIP) of MRD cells. Given the crucial prognostic significance of MRD cells, we addressed genome-wide gene expression in blasts persisting after one week of induction therapy. In spite of the heterogeneous clinical features of the patients, we were able to determine a set of 310 genes whose expression was commonly changed between day 8 and day 0 with an estimated false discovery rate of 0.05. The identified set of genes indicated inhibited cell cycling, reduced metabolism, and expression changes of multiple factors related to B-cell differentiation. These changes collectively suggest that gene expression in day 8 blasts is shifted towards resting mature B cells. Direct comparison of gene expression in blasts and normal B cells and investigation of B-cell differentiation markers on protein level supported this finding. In addition, we observed differential impairment of the key components of the trans-



Monitoring and characterization of MRD cells in acute leukemia. **A:** Detection of MRD cells by multiparametric flow cytometry. **B:** Gene expression in MRD cells displays inhibited cell cycling and expression changes of multiple factors related to B-cell differentiation. **C:** Principal component analysis of gene expression in normal B cells and in leukemic blasts at diagnosis (d0) and under therapy (d8) indicates a shift towards normal B cells in MRD (d8) cells.

lational machinery which correlated with the rates of cytoreduction in peripheral blood. Taken together, investigation of leukemia cells persisting during therapy identifies common and individual expression changes which may potentially affect sensitivity towards anti-leukemic agents and offers new insights into the mechanisms of therapy resistance in ALL.

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Cancer Genetics and Cellular Stress Responses

Clemens A. Schmitt

Scope of the research program

Defective cellular growth control is a pivotal feature of the malignant cell. Driven by mitogenic oncogenes, transformed cells select for mutations that disable ultimate failsafe programs such as apoptosis or cellular senescence. Importantly, DNA damaging anticancer agents may sensitize to programmed cellular responses that overlap with anti-oncogenic failsafe mechanisms. Hence, inactivation of apoptotic pathways may, for example, not only facilitate tumor development but might also simultaneously co-select for resistance to anticancer therapy. Dissecting the pathways that control and execute apoptosis and senescence is expected to identify critical regulators that may be targeted with novel therapies.

Selected scientific findings

Cellular senescence is a p53-controlled program that contributes to the outcome of anticancer therapy in vivo

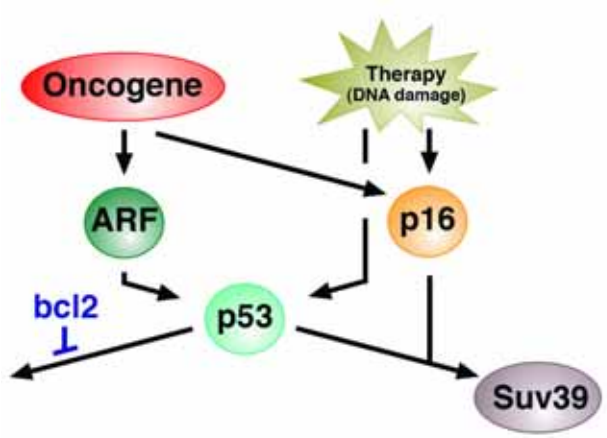
We have utilized transgenic mouse models which are prone to hematological malignancies to study genetics of tumor development and treatment responses. Using the *Eμ-myc* transgenic mouse as a model system, we generated mice – via intercrossing and retroviral gene transfer – that bear lymphomas with defined genetic defects. Bcl2-overexpressing lymphomas appear to be highly protected against the cytoreductive effect of anticancer therapy *in vivo*, but mice harboring these tumors live much longer as compared to those bearing p53-deficient lymphomas. We demonstrated (in collaboration with Scott Lowe) that DNA damaging anticancer therapy may, in the absence of drug-inducible apoptosis, acutely execute cellular senescence, a terminal arrest program that mimics 'replicative senescence', and that may re-program cellular capabilities. Although function and fate of senescent cells and their interaction with surrounding non-neoplastic and malignant cells remain to be elucidated, *in vivo*-analyses identified drug-inducible senescence as a p53 and p16INK4a controlled program that prolongs host survival when tumors are resistant to treatment-induced apoptosis. In turn, repeated exposure of Bcl2-expressing tumors to chemotherapy may select for p53 inactivation, resulting in highly resistant malignancies lacking both a drug-inducible apoptosis and senescence response.

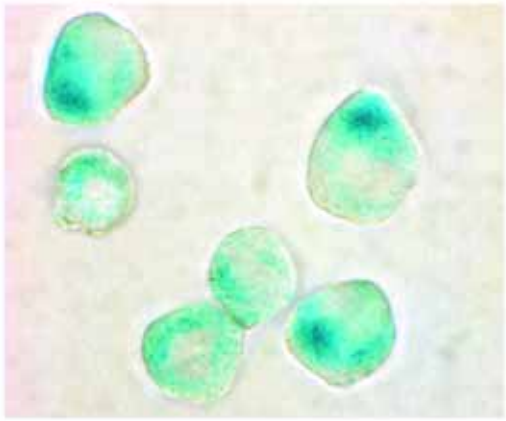
Molecular cytogenetics may refine the prognostic value of genetic lesions to predict treatment outcome

As previously reported, p53 and *INK4a/ARF* mutations promote tumorigenesis and drug resistance, at least in part, by disabling apoptosis and cellular senescence. In a subsequent study, we applied several genome wide approaches to primary lymphomas arising with distinct *INK4a/ARF* lesions to obtain additional genomic prognosticators of treatment outcome. Indeed, using spectral karyotyping (SKY), comparative genomic hybridization (CGH), and fluorescence in situ hybridization (FISH), we find recurrent genomic alterations that refine the predictive value of *INK4a/ARF* lesions on drug responses *in vivo*. Moreover, we identified cytogenetic markers in untreated tumors that were indicative of subsequent mutations during therapy. These data illustrate how genomic information from human tumors may complement established prognostic markers and may improve anticancer treatment strategies.

Inactivation of the Suv39h1 histone methyltransferase promotes Ras-initiated lymphoma formation by disabling cellular senescence

Acute induction of oncogenic Ras provokes cellular senescence involving the retinoblastoma (Rb) pathway but the role of cellular senescence as a tumor suppressive program active *in vivo* remained to be demonstrated. Recently, Rb-mediated silencing of growth-promoting genes by histone H3 lysine 9 methylation (H3K9me)-associated heterochromatin formation was identified as a critical feature of cellular senescence, which may depend on the histone methyltransferase Suv39h1. We have now shown that Suv39h1 governs a senescence response which blunts the oncogenic potential of activated Ras in primary lymphocytes. In turn, Eμ-*N-Ras* transgenic mice harboring targeted lesions at the *Suv39h1*, or the *p53* locus for comparison, succumb to invasive lymphomas. Suv39h1-deficient lymphoma cells grow rapidly but, unlike p53-deficient cells, remain highly susceptible to drug-induced apoptosis. Hence, H3K9me-mediated senescence is a novel Suv39h1-dependent tumor suppressor mechanism whose inactivation permits the formation of aggressive but apoptosis-competent lymphomas in response to oncogenic Ras. Future work is aimed at dissecting the signaling cascades to apoptosis and cellular senescence in greater detail in order to identify molecular lesions as potential targets for novel therapeutic approaches utilizing these programs independent of the devastating effects of broadly DNA damaging compounds.





Drug-senescent Ras-driven T-cell lymphoma cells
(staining for senescence-associated β-galactosidase activity)

Oncogenic signaling, DNA damaging therapy and cellular failsafe responses. Model of cellular pathways leading to apoptosis or cellular senescence upon oncogenic activation and anticancer treatment (left). Cytospin preparation of primary T-cell lymphoma cells that bypassed a Ras-provoked senescence block but that retain the ability to enter drug-inducible senescence as visualized by perinuclear blue staining indicating senescence-associated β-galactosidase activity in repsonse to adriamycin treatment (right).

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Clinical and Molecular Oncology

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Scope

Cell death and cell cycle deregulation in cancer and resistance to anticancer therapy

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 with chemotherapeutic drugs or ionising irradiation and poor survival. Such analyses 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 program in solid tumors and leukemias. Recent pharmacogenomic data obtained from these screenings depict that defects in central regulatory genes (e.g. of the p53 pathway) do not result in global resistance to therapy but may be overcome by adequate therapeutic modalities. Functional consequences of such cell death and cell cycle defects are analysed *in vitro*, often by the use of adenoviral gene transfer for complementation of disrupted genes. In addition, these systems are exploited to gain insights into novel aspects of cell cycle and cell death regulation and their intricate interactions.

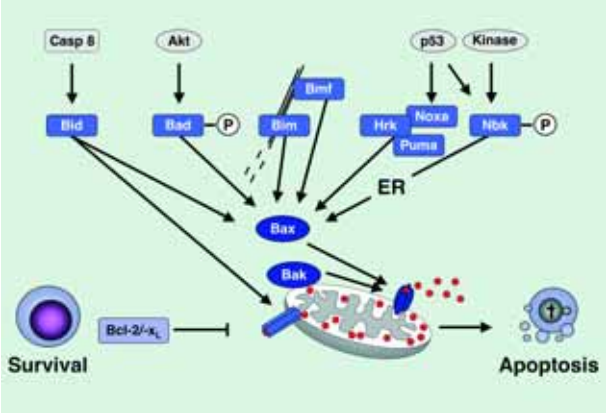
Understanding resistance to anticancer therapy

Anticancer therapies (i.e. chemotherapy and ionising irradiation) 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. While there is few doubt that p53 disruption can serve as a key step during tumorogenesis, there is so far only controversial evidence that p53 by itself is a key factor in the development of clinical resistance to anticancer therapies. To this end, 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. There, in both *in vitro* and in clinical settings, we established that disruption of the intrinsic apoptosis pathway results in resistance to anticancer therapy. We also found that inactivation of single regulatory genes is often insufficient to explain resistance phenomena. In contrast, clinical resistance to anticancer therapy and poor prognosis often arise when cell cycle and cell death signaling is impaired by multiple defects, e.g. upon disruption of p53 and Bax or APAF-1 or Bax and Rb pathway components. 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

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. 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 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. The focus is on the mechanism of activation of pro-apoptotic

Figure 1. Function of BH3-only proteins as death sensors.



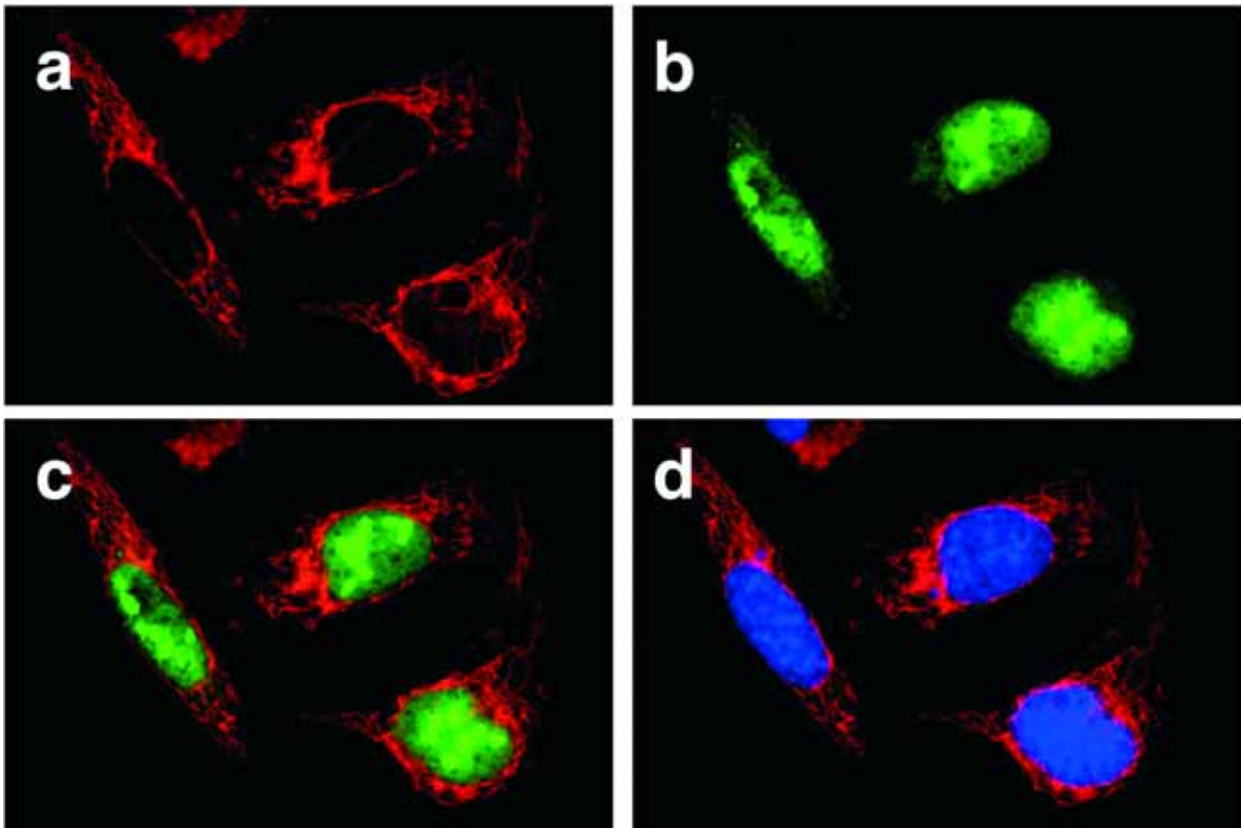


Figure 2. Adenoviral expression of p14ARF
A: Mitochondria are labeled red by stable expression of a DsRed fusion protein as an organelle marker. **B:** p14^{ARF} expression is found in the nucleus and the nucleoli. **C:** Overlay for p14^{ARF} and mitochondria. **D:** Overlay for red labeled mitochondria and DAPI. This experiment shows that p14^{ARF} must trigger the mitochondrial apoptosis pathway via an indirect mechanism. This is unlike the case of p53, which had been described recently to localize not only to the nucleus but also to the mitochondria where p53 exerts transcription independent apoptotic functions.

multidomain Bcl-2 homologs and their interaction with BH3-only proteins that link various upstream signals including death receptors and DNA damage signaling to the mitochondrial and the ER pathway.

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. P14ARF 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 p14ARF induces cell death. Notably, we established that the induction of mitochondrial apoptosis by p14^{ARF} is entirely independent from p53 and Bax. Whereas loss of Bax is functionally complemented by the Bax-homolog Bak and vice versa, the combined loss of both Bax and Bak strongly attenuates p14ARF-induced mitochondrial activaation. However, a substantial proportion of p14^{ARF}-induced apoptosis occurs in a Bax/Bak-independent manner and, therefore, may involve other, so far unexplored, apoptosis signaling pathways. In contrast to apoptosis induction, the triggering of a G1 cell cycle arrest (and presumably premature cellular senescence) by p14^{ARF} is entirely dependent of 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 strongly enhances apoptosis induction by p14^{ARF}. In the same vein, loss of 14-3-3σ or of both p21 and 14-3-3σ strongly augments p14^{ARF}-induced apoptosis. 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.

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Molecular Immunotherapy

Antonio Pezzutto

Our group focuses on the following topics: 1) clinical studies using dendritic cells to induce tumor-specific immune response; 2) analysis of the natural immunity against the adenocarcinoma-associated EpCAM antigen in cancer patients and evaluation of heteroclitic peptides as tools for improving the immune response; 3) gene transfer and DNA vaccination for induction of tumor specific immunity; 4) evaluation of graft versus leukemia reactivity in patients treated with bone marrow/stem cell transplantation and 5) evaluation of T-cell receptor regulation in autoimmunity and cancer.

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

Jörg Westermann, Tuan Duc Nguyen, and J. Kopp.

We have shown that T cells from both normal individuals and chronic myeloid leukemia (CML) patients can specifically recognize the bcr-abl fusion peptide that is characteristic of CML. We have performed a clinical vaccination study using *in vitro*-generated, bcr-abl positive dendritic cells (DC). A reduction in the tumor load has been seen in some vaccinated patients so a follow-up study is being planned in patients who have incomplete remission upon treatment with the abl-inhibitor imatinib. Other CML-related antigens identified in leukemic cells by mass spectrometry of MHC-eluted peptides are being evaluated for immunogenicity. In renal carcinoma, two different studies are performed: HLA-A2 positive individuals are being offered vaccination with a gene-modified tumor cell vaccine that expresses co-stimulatory molecules and secretes Interleukin-7 while patients with other HLA-haplotypes are being offered vaccination with autologous tumor lysate loaded onto partially HLA-matched allogeneic DC. GMP quality DC and gene-modified tumor cells are generated in the GMP laboratory of the Robert Rössle Cancer Clinic.

Induction of T-cell immunity against EpCam (Epithelial Cell adhesion molecule) and evaluation of heteroclitic peptides as tools for improving the immune response

Oliver Schmetzer, in cooperation with P. Schlag (MDC group, Surgical oncology) and K. Falk and O. Rötzschke (MDC group, Cellular immunology of autoimmune reactions).

The epithelial adhesion molecule, EpCam, is overexpressed in human adenocarcinomas. Induction of autoantibodies against EpCam is an infrequent event occurring in a minority of patients with tumors. Some patients have circulating CD4 positive T cells that recognize MHC-II binding EpCam peptides. Since multimere peptides of MHC-II epitopes can amplify the natural occurring immune response, we are evaluating 4-mer and 16-mer EpCam multimeres in cooperation with K. Falk and O. Rötzschke. Moreover, heteroclitic peptides with minor aminoacid substitutions have been generated. It appears that some of these peptides provide a much stronger stimulation of the T-cells by inducing an upregulation of the TCR (s. also chapter below).

Regulation of T-cell receptor (TCR) expression in autoimmunity and cancer

O. Schmezter in cooperation with R.J. Pires (NMR protein structure group, Universidade Federal do Rio de Janeiro, Brasil)

We identified a new splice variant of CD3delta which seems to be a main regulator of TCR surface expression. It codes for a 45-mer polypeptide which has features of a soluble protein. When added to T cell cultures *in vitro*, this polypeptide can induce a marked sustained increase of T-cell receptor density on the cell surface. In some autoimmune conditions, it appears that this variant is largely expressed (e.g., in fluid of inflamed arthritic joints). A number of cytokines and chemokines as well as surface molecules appear to be influenced by this small protein. Further characterization is ongoing.

DNA Vaccination

Jörg Westermann, Gerd Baldenhofer and Tam Nguyeng Hoay in cooperation with U. Höpken and M. Lipp (MDC group, Differentiation and growth control in lymphocyte development)

In order to increase the presentation of putative tumor antigens by antigen presenting cells, we have evaluated cytokines such as flt-3 Ligand (which can induce *in vivo* expansion and recruitment of dendritic cells) and chemokines such as CCL19(ELC) and CCL21(SLC) (cooperation with the MDC group, Molecular Tumor Genetics, M. Lipp) as adjuvants for DNA vaccination. Flt-3L seems to have limited efficiency in generating an improved T cell response. Encouraging results are being obtained with coexpression of the CCR7 ligand CCL19(ELC) which is able to augment the tumor-protective effect of a DNA vaccine by amplifying a TH1-polarized T cell response.

Evaluation of T-cell reactivity against self-antigens and minor histocompatibility antigens in patients receiving allogeneic stem cell/bone marrow transplantation.

Corinna Lenng in cooperation with Prof. R. Arnold (Department of Hematology Oncology, Charité Campus Virchow Klinikum)

Allogeneic bone marrow/stem cell transplantation is currently the only curative treatment option for a number of malignant diseases of the blood system. The tumor responses achieved by this therapy depend to a significant extent on an immunologically mediated **graft-versus-leukemia (GVL)** response. Evaluation of the T-cell responses against leukemia associated antigens and so-called “minor Histocompatibility antigens” is being performed at different times after transplantation and correlated with the chimerism state of the patients.

Selected Publications

Westermann J, A van Lessen, C Schlimper, G Baskaynak, P le Coutre, B Dörken, A Pezzutto (2005) Simultaneous cytokine analysis by Cytometric Bead Array (CBA) allows more sensitive detection of Leukemia-reactive T cells in patients with chronic myeloid leukemia. Br J Haematol (in press)

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Molecular Immunology and Gene Therapy

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The major topics of the group are in the field of cancer immunology and gene therapy. In the last 2 years, the group analysed the role of tumor stroma as a target for T-cell mediated tumor rejection. Furthermore, experimental models were established to analyse spontaneous immune responses against primary non-transplanted tumors.

CD8 effector T cells reject tumors by direct antigen recognition but indirect action on host cells

CD8 effector T cells recognize malignant cells by monitoring their surface for the presence of tumor-derived peptides bound to MHC class I molecules. In addition, tumor derived Ags can be cross-presented to CD8 effector T cells by APCs. IFN-γ production by CD8 T cells is often critical for tumor rejection. However, it remained unclear as to whether 1) CD8 T cells secrete IFN-γ in response to Ag recognition on tumor cells or APCs and 2) whether IFN-γ mediates its antitumor effect by acting on host or tumor cells. We showed that CD8 effector T cells can reject tumors in bone marrow-chimeric mice incapable of cross-presenting Ag by bone marrow-derived APCs and that tumor rejection required host cells to express IFN-γ-receptor. Together, CD8 effector T cells recognize Ag directly on tumor cells, and this recognition is sufficient to reject tumors by IFN-γ acting on host cells.

Tumor-induced antibodies resemble the response to tissue damage and do not indicate T cell competence

Tumor-associated antibodies are frequently detected in cancer patients. In order to determine whether the recognized antigens are rejection antigens, we screened a cDNA expression library of the mouse TS/A tumor with TS/A-immune serum and isolated 8 IgG-reactive clones, representing self-antigens that were expressed in normal tissues and other tumor lines. Three of the antigens had previously been identified in humans by this cloning strategy. None of the antigens revealed to be a rejection antigen in normal mice demonstrated by an otherwise effective plasmid immunization. For one of the identified antigens, a-catenin, it has been shown that the induction of IgG antibodies by protein immunization does not correlate with tumor rejection. For another antigen, vimentin, it has been shown that vimentin-deficient mice, but not vimentin-competent mice, reject vimentin-expressing tumors indicating T cell tolerance despite the fact that tumor cell immunization induces anti-vimentin IgG antibodies. Tissue damage induced by adenovirus infection induced an antibody response similar to tumor cell immunization, exemplified by 2 of the antigens. We conclude that the tumor-induced antibodies mirror tissue damage and that the antibody-inducing antigens can serve as rejection antigens if they are recognized as foreign.

CY15, a malignant histiocytic tumor that is phenotypically similar to immature dendritic cells

The origin and pathogenesis of histiocytic malignancies and the biology of the tumor cells are poorly understood. We have isolated a murine histiocytic tumor cell line (CY15) from a BALB/c IFNγ$^{-/-}$ mouse and characterized it in terms of phenotype and function. The morphology, as judged by electron microscopy, and the surface marker phenotype suggests that CY15 cells are similar to immature dendritic cells (CD11c^{low}, MHC II low, CD11b^{+}, B7.1^{+}, B7.2^{+}, and CD40^{+}). The cells form tumors in BALB/c mice and metastasize to spleen, liver, lung, kidney, and, to a lesser extent, to lymph nodes and bone marrow, as judged by the growth of green fluorescent protein transfected tumor cells in mice. CY15 cells are capable of actively taking up antigen (FITC-ovalbumin) and can stimulate T lymphocytes in an allogenic mixed lymphocyte reaction but less effectively than their normal counterparts (immature dendritic cells). They respond to interleukin 4 (IL-4) with the up-regulation of CD11c. If stimulated with IFNγ, the cells up-regulate MHC II, CD40, B7.1, and B7.2. Lipopolysaccharide induces the cells to up-regulate B7.1 and B7.2 and to secrete TNFα and IL-12. Based on these data, CY15 is a dendritic cell–like tumor cell line and may serve as a transplantable tumor model for histiocytosis in humans.

Dual T cell receptor T cells with two defined specificities mediate tumor suppression via both receptors

Grafting T cells with new antigen specificity by T cell receptor (TCR) gene transfer could greatly facilitate adoptive T cell immunotherapy. Little is known about how two TCR on one T cell influence each other. Among other reasons, this is due to the fact that only one TCR specificity is known. We have genetically generated murine dual TCR T cells (OT-I/P14), specific for ovalbumin (ova257) and lymphocyte choriomeningitis virus glycoprotein (gp33). These cells retain both specificities and can be stimulated by either antigenic peptide to proliferate and produce IFN-γ. Even though one TCR (P14) is expressed at reduced levels on dual TCR T cells, the peptide sensitivity of these cells is similar to that of single TCR T cells of the same specificity. TCR down-modulation on dual TCR T cells depends primarily on binding of the specific ligand.

Adoptively transferred dual TCR T cells suppress the growth of both B16-ova and B16-gp33 melanoma cells, regardless of the peptide used for in vitro activation. Taken together, despite a certain dominance of expression between two TCR on the same T cell, this does not necessarily have functional consequences.

Sporadic immunogenic tumors induce T cell tolerance

The recognition and elimination of tumors by T cells, a process termed cancer immunosurveillance, is effective against certain virus-associated cancers. Spontaneous tumors often induce a specific immune response and are, therefore, also immunogenic. However, it is not clear whether they can be controlled by T cells. The immunosurveillance hypothesis postulates that tumors, if they eventually grow, escaped T-cell recognition by losing immunogenicity. Generating a mouse model of sporadic cancer based on rare spontaneous activation of a dormant oncogene, we showed that immunogenic tumors do not escape their recognition but induce tolerance. In this model, tumors derive from single cells and express a tumor-specific transplantation rejection antigen. Whereas vaccinated mice remain tumor-free throughout their lifetime, naive mice always develop a progressively growing tumor. We also showed that despite specific recognition by T cells, the tumors do not lose their intrinsic immunogenicity and are rejected after transplantation in T cell-competent recipients. Furthermore, in the primary host, tumor-induced tolerance is associated with the expansion of non-functional T cells. Together, our data argue against immunosurveillance of spontaneous cancer. These data are in line with results showing that the absence of T-cells or perforin does not significantly increases tumor incidence by the chemical carcinogene methlcholanthron.

Selected Publications

Schüler, T. and Blankenstein, Th. (2003). $CD8^+$ effector T cells reject tumors by direct antigen recognition but indirect action on host cells. J. Immunol. 170: 4427-4431.

Kammertoens, T., Willebrand, R., Erdmann, B., Li, L.P., Li, Y.P., Engels, B., Uckert, W., and Blankenstein, Th. (2005). CY15, a malignant histiocytic tumor that is phenotypically similar to immature dendritic cells. Cancer Res. 65:2560-2564.

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Gladow, M., Uckert, W. and Blankenstein, Th. (2004). Double T cell receptor T cells with two defined specificities mediate tumor suppression via both receptors. Eur. J. Immunol. 34: 1882-1891.

Willimsky, G. and Blankenstein, Th. (2005) Sporadic immunogenic tumors avoid destruction by inducing T-cell tolerance. Nature 437:141-146.

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Molecular Cell Biology and Gene Therapy

Wolfgang Uckert

The focus of our group lies on the adoptive transfer of gene-modified T cells for cancer therapy. We address questions related to the optimisation of retroviral vectors, the generation of T cells with new specificities by T cell receptor (TCR) gene transfer, the adoptive transfer of genetically modified T cells in mice, and related safety aspects.

Retroviral vectors derived from Moloney murine leukaemia virus (MLV) are commonly used to transfer foreign genetic information into T cells. We could show that retroviral vectors carrying the envelope of the murine leukaemia virus 10A1 transduced primary human T cells most efficiently. However, when using these vectors, the expression level of the transferred genes is often unsatisfactory. We therefore analysed different *cis*-regulatory elements in the context of retroviral vectors. Compared to those elements used in MLV-based vectors, the *cis*-regulatory elements in the murine myeloproliferative sarcome virus-based vector MP71 yielded an up to 75-fold increase in transgene expression in T cells.

Using the MP71 vector, the α and β chains of a TCR that recognises a tumour antigen on renal cell carcinoma (RCC) cells was transferred into T cells. The TCR molecule was expressed on the cell surface of primary human T cells of different donors. TCR gene-modified T cells could be stimulated in an antigen-specific and HLA-restricted fashion and led to the recognition and lysis of RCC cells. The magnitude of induced effector functions (cytokine secretion and tumour cell lysis) was comparable to RCC-stimulated cells of the original T cell clone from which the TCR genes were isolated. Currently, we are investigating the transfer of specificities for a RCC tumour-associated antigen, NY-ESO-1, and Epstein-Barr virus antigens. First results indicate the general applicability of the MP71 vector for high-expression gene transfer in T cells. Thus, the redirection of T cells by TCR gene transfer could greatly facilitate adoptive transfer by generating T cells with anti-tumour specificity.

In order to further improve TCR-transfer, we developed a simplified and accelerated method for the generation of high-titre TCR-retrovirus in a suspension packaging cell line. The virus producing cells, based on a TCR-deficient T cell line, can be enriched via the expression of the virally encoded TCR, analogous to the enrichment of packaging cells for fluorescent marker-retroviruses. This approach circumvents the very tedious method of screening for high-virus producing packaging cell clones.

Regarding the safety of retroviral transfer of TCR genes into T cells, several aspects are being addressed. Little is known about how two TCRs on one T cell influence antigen recognition, specificity, and tumour cell lysis. In collaboration with T. Blankenstein, we have generated a mouse model of double TCR T cells (OT-I x P14) specific for ovalbumin (ova) and lymphocyte choriomeningitis virus glycoprotein 33 (gp33). These cells retain both specificities and can be activated by triggering either TCR with its cognate peptide. Adoptively transferred double-TCR T cells suppress the growth of both B16-ova and B16-gp33 murine melanoma cells regardless of the peptide used for prior *in vitro* T cell activation. Another feature of double-TCR T cells is the surface expression of chimeric TCRs originating from TCR-chains of endogenous and exogenous origin. We have shown that these chimeric TCRs arise after crossing TCR transgenic mice and also after T cell transduction with TCR-retroviruses. Using siRNA technology, we want to suppress the expression of the endogenous TCR and, thereby, circumvent potential safety concerns related to dual-TCR T cells and chimeric TCRs.

Finally, the potential of in vivo antibody depletion of TCR-redirected T cells is being analysed. So far, we have introduced a tag into the transferred TCR which can be used as a target for depletion in case of the undesired occurrence of a lymphoproliferative disease or the induction of graft versus host disease after transfer of TCR gene-modified T cells.

Selected Publications

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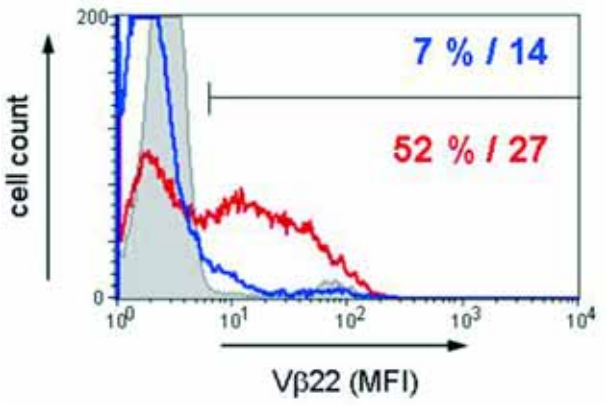

Figure 1
The β-chain of the retrovirally encoded RCC-specific TCR is expressed on MP71-tcr-transduced primary human T lymphocytes. TCR β-chain surface expression is visualised by staining non-transduced cells (grey shade), L-tcr-transduced (MLV vector, blue line), and MP71-transduced cells (MP71 vector, red line) with a PE-conjugated anti-Vβ22 mAb and subsequent flow cytometry analysis. Indicated are % Vβ22-positive cells and the mean fluorescence intensity (MFI) of these positive cells.

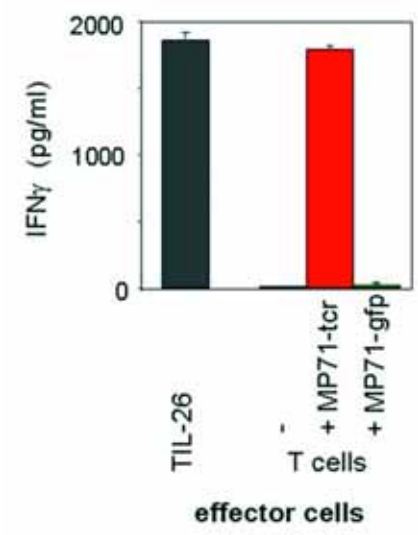

Figure 2
MP-tcr-transduced primary T cells and tumour infiltrating T cell clone TIL-26 secrete similar amounts of interferon-γ upon stimulation with the cognate tumour cell line RCC-26. Cytokine was detected in supernatant of a 24 h coculture of RCC-26 and effector cells as indicated (1:5 effector to target ratio) by ELISA.

Engels, B, Cam, H, Schüler, T, Indraccolo, S, Gladow, M, Baum, C, Blankenstein, T, Uckert, W. (2003). Retroviral vectors for high-level transgene expression in T lymphocytes. Hum Gene Ther. 14, 1155-1168.

Patent Application

Uckert, W. Blankenstein, T. (2005). Construction of a T cell line-based packaging cell line for the production of T cell receptor retroviruses by enriching of CD3 expressing cells. 05013833.8 EPC.

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Cellular Immunology of Autoimmune Reactions

Kirsten Falk
Olaf Rötzschke

The driving force in the progression autoimmune diseases are autoreactive T cells. In most cases, these autoreactive T cells are $CD4^+$ T cells that have escaped the tolerance control mechanisms of the immune system. While they are responsible for the induction of diseases, such multiple sclerosis or diabetes, they can be beneficial when recruited in the context of tumor immunotherapies. The group is currently interested in three basic problems related to the activation and control of these cells:

1. Modulation of the immune response by small molecular 'MHC-loading enhancer' (MLE)

It is already well established that genetic factors, such as expression of certain allelic forms of MHC molecules, play an important role in autoimmune diseases. At least equally important for the induction of these diseases are environmental influences. Up to now, however, these environmental factors are largely unknown. Recent experiments in our group have demonstrated that small organic compounds are able to trigger the exchange of peptide antigens on the surface of activated antigen presenting cells. By interacting with a specific pocket on the class II MHC molecule, the peptide receptor responsible for displaying antigens to $CD4^+$ T cells, they 'open up' the peptide binding site and allow antigens to slip onto the receptor (Fig. 1A). As a consequence, the amount of antigens loaded onto MHC-expressing cells is significantly higher when these catalytic small molecules are present during the loading process (Fig.1B). As 'MHC-loading enhancers' (MLE), these compounds could be used as molecular tools for vaccination or in therapeutic settings. By mediating the transfer of antigens, they can enhance immune responses directed against pathogens or transformed tissue.

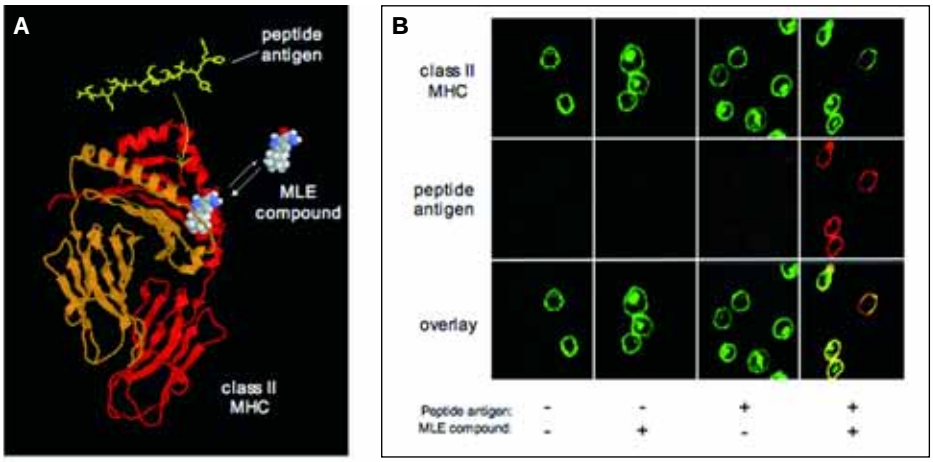
On the other hand, the 'accidental' transfer of autoantigens (peptides and proteins), the target structure of autoreactive T cells, might also lead to the induction of allergy or chronic autoimmune diseases. *In vitro* studies by our group have already shown that myelin-reactive $CD4^+$ T cells, which are responsible for the induction of multiple sclerosis (MS), can be activated by crude preparations of spinal cord when MLE compounds are present. MLE compounds might, therefore, represent a novel class of environmental risk factors and the group is currently defining the structural requirements of the compounds and investigating their impact on experimental autoimmune and tumor model systems.

2. Control of autoimmune reactions by regulatory CD4+ T cells

Several mechanisms have been described which potentially allow to control autoreactive T cells. Besides direct (or "suicidal") mechanisms, such as induction of 'high-zone tolerance', indirect control mechanisms are most promising. Indirect control is mainly accomplished by regulatory T cells, which upon antigen-specific activation 'silence' or eliminate other activated immune cells in their vicinity. $CD25^+$ $CD4^+$ T cells have recently been identified to be one of the subpopulations responsible for this effect. Another subset are IL10 producing CD25-CD4 T cells. Both subsets are currently under investigation. Some of the specific experimental tools employed in these studies are T cell epitope oligomers (repetitive T cell antigens), which have been found to be effective inducers of antigen-specific tolerance in vivo. The primary goal of these studies is the exploration of ways allowing a specific recruitment or inactivation of these cells for the treatment of autoimmune diseases and cancer, respectively, the identification of key genes responsible for differentiation and maintenance of the suppressor status of regulatory T cells, and the characterization of the functional role of regulatory T cell subsets.

3. Selective activation of auto-reactive CD4 effector T cells in tumour model systems

While in autoimmune diseases the action of autoreactive $CD4^+$ T cells is often fatal, it can be beneficial in the context of tumour immune-therapies. The damage inflicted by these cells is very specific and restricted to the tissue expressing the autoantigen. Furthermore, the immune response of autoreactive $CD4^+$ T is usually chronic and often leads to the recruitment of other immune cells, such as cytotoxic $CD8^+$ T cells or B cells, which also participate in the tissue-specific elimination and removal of cells. By using model antigens derived from human cancer diseases, such as BCR/ABL (leukaemia) and NY-ESO-1 (melanoma), the group is trying to adapt proinflammatory conditions typical for chronic autoimmune diseases by addressing tumor-specific $CD4^+$ T cells. The use of transgenic mouse models expressing human class II MHC molecules permits rapid exchange of experimental data, antigens, and reagents with corresponding *ex vivo* experiments with cancer patients and ensures the efficient transfer from 'bench to bedside'. The trials employ antigens with enhanced immunogenicity, such as epitope oligomers or lipo-peptides, as well as MLE compounds and include the construction of inducible animal model systems (TET system). Results obtained within the two other focal points of our research - small molecular ligand exchange catalysts and immune-regulation - are implemented in these trials to achieve additional leverage for the generation of a proinflammatory environment that will promote productive tumor-specific immune responses.



Effect of 'MHC-loading enhancer' (MLE) compounds.
(A) Putative mechanism of MLE-mediated antigen loading. By docking to a specific pocket on the class II MHC molecule they induce and stabilize a 'peptid-receptive' conformation that allows antigens to enter the open peptide binding site.
(B) MLE-mediated antigen-loading of cell surface MHC. Cells expressing class II MHC molecules were incubated with a peptide antigen in the absence or presence of an MLE compound. After the incubation the cells were analyzed by confocal laser scanning microscopy to visualize the amount of peptide antigen loaded onto the cell surface.

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Burster, T., Beck, A., Tolosa, E., Marin-Esteban, V., Rotzschke, O., Falk, K., Lautwein, A., Reich, M., Brandenburg, J., Schwarz, G., et al. (2004). Cathepsin G, and not the asparagine-specific endoprotease, controls the processing of myelin basic protein in lysosomes from human B lymphocytes. J Immunol 172, 5495-5503.

Marin-Esteban, V., Falk, K., and Rotzschke, O. (2003). Small-molecular compounds enhance the loading of APC with encephalitogenic MBP protein. J Autoimmun 20, 63-69.

Patent Applications

File number 10 200 40 44 556.2 (German Patent Office)
File number 10 200 40 54 545.6 (German Patent Office)

Falk, K., Rötzschke, O. (2004) Katalysatoren der Beladung von MHC Molekülen *(Small molecular catalysts of MHC loading)*

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Experimental Pharmacology

Iduna Fichtner

The group is mainly interested in the establishment of preclinical models for the testing of novel anticancer agents and for investigations concerning the in vivo potential of stem cells. Our research is focused preferentially on the following three projects:

- Molecular characterization of breast carcinomas
- Development of a novel anti-metastatic agent
- Engraftment and organ-specific differentiation of adult stem cells

Molecular characterization of breast carcinomas

Based on long-standing experience with hormone dependent breast cancer, we were interested in mechanisms of tamoxifen resistance. One patient-derived xenograft, MaCa 3366, showed a high response rate towards anti-estrogen. From this xenograft line, a resistant subline, MaCa 3366/Tam, was developed in a clinically adapted manner. Tumour material from both xenograft lines was used for a molecular characterization on RNA and protein level. Results of Affymetrix microarrays revealed that more than 100 transcripts are changed in abundance between the resistant and sensitive line. Among these are several interferon-inducible and estrogen-responsive genes as well as genes involved in breast carcinogenesis. The genes neuronatin and bone marrow stem cell antigen 2 were distinctly upregulated in MaCa 3366/Tam. In addition, protein analysis was performed by two-dimensional gel electrophoresis followed by ESI mass spectrometry and databank comparison of differentially expressed molecules. We have identified 21 proteins being at least two-fold differently expressed between the sensitive and the resistant breast cancer xenografts. The biological functions of these proteins are related to cell-cell adhesion and interaction, signal transduction, DNA and protein synthesis machinery, mitochondrial respiratory chain, oxidative stress processes, and apoptosis. Both defined genes and proteins, which are distinctly expressed in relation to tamoxifen response, will be further evaluated as potential targets for diagnostic or therapeutic approaches.

Development of a novel anti-metastatic agent

One decisive step within the cascade of metastasis is the formation of aggregates between tumor cells and blood cells within the vasculature and their subsequent adherence to the endothelial membrane. We found using *in vitro* investigations that the aggregation behavior of platelets with HT-29 colon carcinoma cells could be influenced by the addition of surface modified (sialyl Lewisx) liposomes.

The metastasis pattern was changed in nude mice xenotransplanted with HT-29 cells. While in control mice, metastases in lung, liver, and in intestine were prevailing, liposomal treatment completely prevented lung metastases but resulted in muscle metastases. Further studies are planned to elucidate the detailed mechanism of interaction between tumour and blood cells and to develop an agent for therapeutic intervention.

Engraftment and organ-specific differentiation of adult stem cells

We continued studies with cord blood derived adult stem cells and focused especially on their potential for liver-specific differentiation. Stem cells were incubated with stem cell factor (SCF), hepatocyte growth factor (HGF), or a combination of both. SCF alone or in combination with HGF clearly stimulated stem cell proliferation *in vitro*. The expression of CD11a (integrin LFA-1) and CD44 (leukocyte homing factor) increased in the presence of SCF. The combination of SCF and HGF clearly enhanced the expression of cytokeratin 8/18, an epithelial marker which is also expressed in hepatocytes. Transplantation of cytokine-stimulated stem cells into NOD/SCID mice revealed an increase of engraftment after extended periods of time (28 to 55 days) in bone marrow, liver, and spleen. The number of human cells in these organs was not decisively influenced by cytokine pre-treatment of cells. In contrast, liver injury (carbon tetrachloride administration) in NOD/SCID mice increased the rate of stem cell homing to the liver while the additional systemic administration of HGF increased bone marrow and spleen engraftment. In the future, we are especially interested in potentially triggering stem cell behavior into a tissue or organ-specific differentiation and will directly compare the behavior of adult and embryonic stem cells.

Selected Publications

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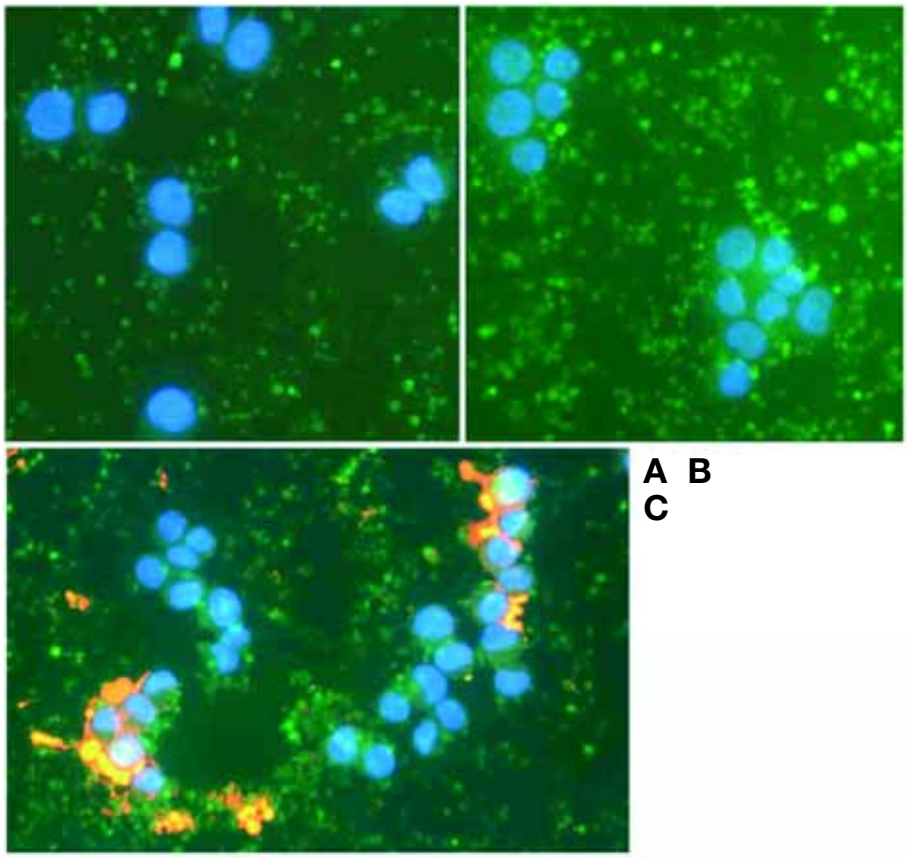
Becker, M, Sommer, A, Krätzschmar, JR, Seidel, H, Pohlenz, H-D, Fichtner, I. (2005). Distinct gene expression patterns in a tamoxifen-sensitive human mammary carcinoma xenograft and its tamoxifen-resistant subline MaCa 3366/TAM. Mol. Cancer Therap. 4, 151-168.



Complex formation of tumour cells in the presence of platelets and liposomes
Fluorescence microphotographs of mixtures of platelets (calcein-AM labelled, green), HT29 colon carcinoma cells (Hoechst 33258 labeled, blue) and liposomes (rhodamine-PE-labeled, red (magnification 63x). **A:** Tumor cells and platelets without activation; **B:** Tumor cells and platelets after activation with 0.01 units thrombin; **C:** Tumor cells and platelets after activation in the presence of sLe^X- liposomes.

Besada, V, Diaz, M, Becker, M, Ramos, Y, Castellanos-Serra, L, Fichtner, I. (2005). Proteomics of xenografted human breast cancer indicates novel targets related to tamoxifen resistance. Proteomics, in press.

Zeisig, R, Stahn, R, Wenzel, K, Behrens, D, Fichtner, I. (2004). Effect of sialyl Lewis X-glycoliposomes on the inhibition of E-selectin-mediated tumour cell adhesion in vitro. Biochim. Biophys. Acta 1660, 31-40.

Keil, C, Zeisig, R, Fichtner, I. (2005). Effect of surface modified liposomes on the aggregation of platelets and tumor cells. Thrombosis and Haemostasis, 94, 404-11

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Bioethics and Science Communication

Christof Tannert

Relevant Topics: embryonic cells, stem cells, gene therapy, the changing notion of disease and illness, and pathogenomics.

Delphi Study

The study was finalized in 2004 and shows trends and time horizons as well as basic parameters and important local/national conditions for stem cell research.

Online-Conference

An Online-Conference of relevant stakeholders of the social, ethical, and economic aspects of biomedicine (ELSA) was finalized in 2004. The interface between basic research and its clinical application and the role of the clinician in steering the transfer were highlighted.

Citizens' Conference

A Citizens' Conference on the ELSA problems of stem cell research was held in March 2004. On the basis of several weekend discourses, the group wrote a statement and presented it to the German parliament (Deutsche Bundestag). The citizen's statement and diverse evaluations of this special example of "deliberative democracy" were published (see Tannert, C, and Wiedemann, P (2004, Eds.): Stammzellen im Diskurs: Ein Lese- und Arbeitsbuch. Oekom-Verlag München).

International Summer School on Biomedicine and Ethics

The workshop was conducted over 8 days in September 2004. The focal points were ethical aspects of predictive medicine, especially in the field of breast cancer, and pre-implantation diagnostics. Fourteen (14) French and German senior experts offered lectures and workshops and 25 Students from Poland, France, and Germany participated.

International Congress "Regenerative Medicine and Biopolitics"

With 140 participants from 11 countries, including members of the German government and the European Commission, the conference focused on the ethical, legal, and social aspects (ELSA) of stem cell research in Germany (day 1) and within the European Union (day 2). The event will be documented in an upcoming publication (Editors Franzen/Tannert Akademische Verlagsgesellschaft, Berlin).

Prospective study 'Future Trends and Challenges in Pathogenomics'

As part of the EU-funded ERA-NET project PathoGenoMics, a prospective study was conducted in order to provide an overview of current and future trends and challenges in the field of genomic research on pathogenic microorganisms. We pursued a dual approach, conducting both a survey with national and international experts in the field of pathogenomics and an extensive literature research.

Internet presence

Our group participates in a website (www.bioethik-diskurs.de) with bi-weekly scientific news, interactive discourse-pages, and the Online-Game GenEthix, developed in cooperation with the German Human Genome Project, and the Fachhochschule Potsdam. Monthly site visitors number around 12,000 (September 2005).

Selected Publications

Wiedemann, PM, Simon, J, Schicktanz, S, Tannert, C. (2004). The future of stem cell research in Germany. A delphi study. EMBO Reports 5 (10), 927-931.

Niewöhner, J, Wiedemann, P, Schicktanz, S, Tannert, C. (2005). Participatory prognostics in Germany – developing lay scenarios for the relationship between biomedicine and the economy in 2014, J. Technological Forecasting and Social Change, 72(2), 195-211.

Pompe, S, Bader, M, Tannert, C. (2005). The State of the Art, and the Legal and Public Debate on Stem Cell Research, EMBO Reports, 6 (4), 297-300.

Pompe, S, Simon, J, Wiedemann, PM, Tannert, C. (2005). Future trends and challenges in pathogenomics. A Foresight Study. EMBO-Reports 6 (7), 600-605.

Niewöhner, J, Tannert, C. (2005). Online-Diskurs zu den ethischen Fragen der Biomedizin. Zschr. Biopolitik 4 (1), 53-63.

Discourse on ethical questions of biomedicine (01 GP 0105 & 01 GP 0155)

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Function and Dysfunction of the Nervous System

Pathophysiological Mechanisms of Neurological and Psychiatric Disorders

Imaging of the Living Brain

Signalling Pathways in the Nervous System

Coordinators:
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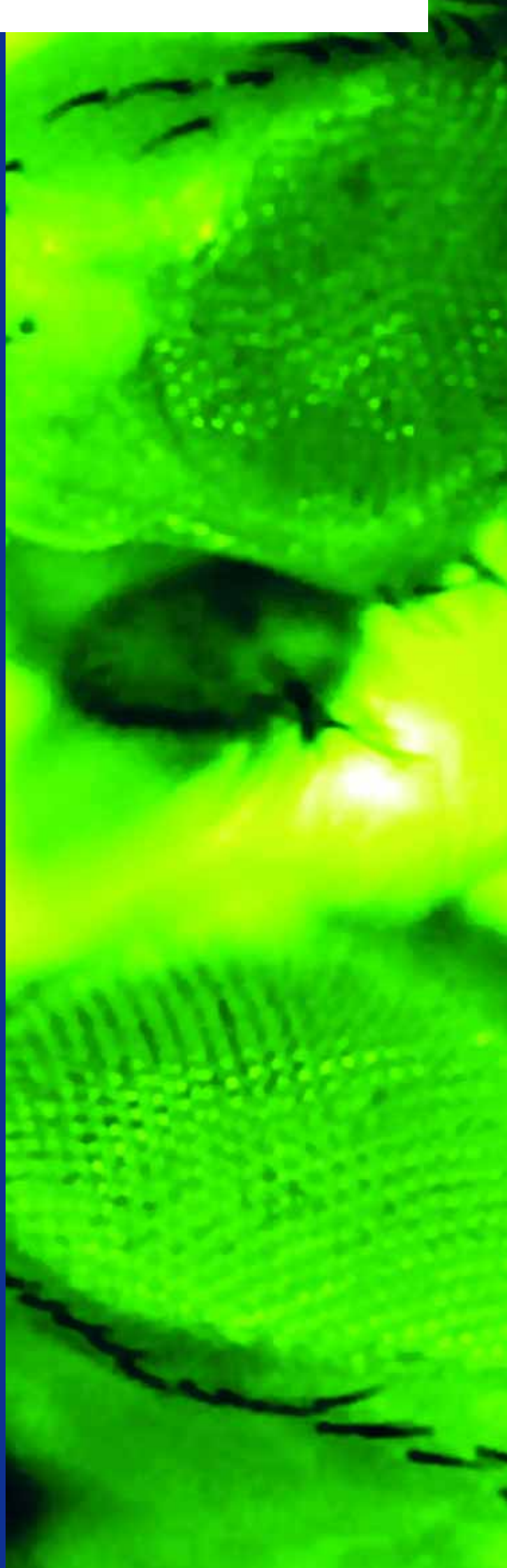


Function and Dysfunction of the Nervous System

Carmen Birchmeier-Kohler
Helmut Kettenmann

The structure and function of the nervous system are very complex. We only partially understand the molecular mechanisms that are used to establish and maintain neurons or glial cells, that control the interactions of these cells in complex neural circuits, and that make it feasible that such circuits process and store information. Recent advances in genomic, proteomic, and imaging technologies now allow for a more systematic analysis and will accelerate the furthering of our knowledge.

The Neuroscience research program at the MDC focuses on molecular and cellular analyses of nervous system development and function. Molecular biology, biochemistry and proteomics, genetics, immunocytochemistry, electrophysiology, imaging techniques, and anatomy are used in the research of the groups. Particular emphasis is given to the analysis of mechanisms that underlie neuropathological conditions.

The data from the human genome sequencing projects have predicted the existence of thousands of new gene products whose functions are unknown. Proteins typically bind to other proteins to exert a function. The identification of protein interactions has been used successfully in the past to infer function and to place proteins into particular pathways and regulatory networks. Erich Wanker's laboratory has now used a systematic approach to define, on a large-scale, protein interactions and interaction networks. This project relies on an automated yeast two-hybrid system and resulted in the generation of the first human interaction map consisting of 3,186 interactions among 1,705 proteins (Stelzl et al., *Cell*, 2005, 122, 957-68). The German National Genome Research Network funded this large-scale project. Large-scale yeast two-hybrid analysis was also used to study protein products relevant for human disease. Thus, the Wanker laboratory has also generated an interaction map for the Huntingtin gene product, whose aggregation is responsible for Huntington's disease. This enabled the identification of GIT1, a G-protein coupled receptor kinase-interacting protein, which enhances the aggregation of Huntingtin (Goehler et al., *Mol. Cell,* 15, 853-65).

Funktion und Dysfunktion des Nervensystems

Carmen Birchmeier-Kohler
Helmut Kettenmann

Die Struktur und Funktion des Nervensystems sind außerordentlich komplex, und wir verstehen bisher nur in Anfängen, wie neuronale Schaltkreise aufgebaut und erhalten werden. Ebenso ist noch unklar, wie Informationen in solchen Netzwerken verarbeitet und gespeichert werden. Technologische Fortschritte und die Verfügbarkeit der Genomsequenz des Menschen sowie von Modellorganismen erlauben nun eine systematischere Analyse und werden uns weitere Einblicke in die Funktionen des Nervensystems ermöglichen. Das Forschungsprogramm Neurowissenschaften am MDC stellt molekulare und zelluläre Analysen in den Vordergrund. Techniken aus der Molekularbiologie, Biochemie, Genetik, Elektrophysiologie, Imaging und Anatomie werden eingesetzt, um die Entwicklung und Funktion des Nervensystems zu verstehen. Ein besonderer Schwerpunkt liegt auf der Analyse der molekularen Mechanismen, die zu Erkrankungen des Nervensystems führen.

Die Auswertung der Sequenz des menschlichen Genoms sagt die Existenz von hunderten von Proteinen voraus, deren Funktionen unbekannt sind. Um eine Funktion auszuüben, binden Proteine typischerweise an andere Proteine, und die Ermittlung von Interaktionspartnern war schon in der Vergangenheit oft mit Erfolg genutzt worden, um ihre Funktionen zu bestimmen. Das Labor von Erich Wanker begann eine systematische Analyse, um im großen Umfang Proteininteraktionen zu entdecken und Proteinnetzwerke zu identifizieren. Dazu nutzte die Gruppe ein automatisiertes Hefe-two-hybrid System und bestimmte eine erste Interaktions-Landkarte menschlicher Proteine: Sie konnten 3186 Interaktionen von 1705 Proteinen aufzeigen (Stelzl et al., *Cell*, 2005, 122, 957-68). Das große und sehr erfolgreiche Projekt wurde im Rahmen des Nationalen Genomforschungsnetzes vom BMBF gefördert. Die gleichen Techniken wurden von der Arbeitsgruppe eingesetzt, um Proteine zu untersuchen, die bei der Entstehung menschlicher Krankheiten eine wichtige Rolle spielen. Das Labor von Erich Wanker konnte so die Interaktionen des Proteins, das vom Huntingtin-Gen kodiert wird, untersuchen; die Aggregation dieses Proteins ist für Chorea Huntington, eine neurodegenerative Erkrankung, verantwort-

Stimulation of neuronal regeneration in patients with neurodegenerative disease or brain cancer might be an interesting therapeutic tool for the future. Helmut Kettenmann's laboratory discovered that highly invasive brain tumours (gliomas) attract neural precursor cells from the stem cell niches of the adult brain into the margin of the tumour. The neural precursors have a direct anti-tumourigenic effect *in vitro* and mediate prolonged survival in an animal model. The natural decline in neural precursors (and, therefore, of anti-tumourigenic action) with increasing age of an individual might indicate a mechanism that contributes to the increased risk in the occurance of glioma in elderly subjects (R. Glass, 2005 *J. Neurosci*. 25:2637-46). Gerd Kempermann's group aims to understand the properties and behaviour of neuronal stem cells. Gerd Kempermann could show that long-term physical exercise in aging mice prevented the normal age-related decline in stem cell proliferation in the adult hippocampus. Interestingly, this increased stem cell activity did not translate into a maintained higher level of adult neurogenesis although the potential for neurogenesis was enhanced.

Fritz Rathjen's group investigates the molecular mechanisms by which electric activity influences the development of synapses. They systematically searched for cell surface proteins that are modulated by neuronal activity. Thus, CALEB was identified, a transmembrane protein that contains an EGF domain. Neuronal activity facilitates the cleavage of CALEB, which exposes its EGF domain. Mutation of the CALEB gene alters the release features of synapses during a critical phase of synapse development. However, the morphology and numbers of synapses remained unchanged in the absence of CALEB. (R. Juttner et al., 2005 Neuron 46, 133-45). These findings indicate that CALEB provides a molecular basis for maintaining normal release probability at early developmental stages.

The sense of touch is not a single sense, since neuroscientists distinguish the perception of texture, temperature, and pain. Distinct neurons located in sensory ganglia innervate the skin and relay this information to the spinal cord. Neurons in the spinal cord integrate and process the sensory information and transmit it to higher brain centres. Sensory neurons that detect stimuli, such as a light brush of the skin or intense, painful heat, are functionally distinct but marker genes whose expression distinguishes them were unknown. The study of pain has been transformed in recent years by the identification of multiple new molecules that play crucial roles in the amplification of pain (or hyperalgesia) following injury. This field was recently reviewed and summarized in an article from Gary Lewin and his colleagues (Lewin et al. 2004 Curr. Opin. Neurobiol. 14, 443-9). In collaboration with Frederique Scamps in Montpellier France, Lewin's group recently showed that sensory neurons with a distinctive Rosette-like morphology in culture could be identified as D-hair mechanoreceptors on the basis of their expression of large T-type calcium currents and an absolute dependence on Neurotrophin-4 (Dubreuil et al. 2004 *J. Neurosci.* 24, 8480-8484). Furthermore, Alistair Garratt has found that mice that are mutant for the tyrosine kinase receptor c-kit are unable to sense pain in an appropriate manner. In addition, the altered properties of the pain processing sensory neurons are currently being studied in collaboration with Gary Lewin.

lich. In diesem Zusammenhang wurde das GIT-Protein identifiziert, das an Huntingtin bindet und die Aggregation des Proteins steigert (Goehler et al., *Mol. Cell* 15, 853-65).

Die Stimulation der Regeneration von Nervenzellen bei Patienten mit neurodegenerativen Erkrankungen oder mit Gehirntumoren könnte in der Zukunft ein interessanter therapeutischer Ansatz werden. Das Labor von Helmut Kettenmann zeigte, dass maligne Tumore des Gehirns (Glioblastome) neuronale Vorläuferzellen anlocken; diese Vorläuferzellen bilden sich aus neuronalen Stammzellen des Gehirns. Neuronale Vorläuferzellen reduzieren das Wachstum der Tumorzellen in Kultur und verlängern, im Versuchstier, die Lebensdauer von Mäusen mit Glioblastomen (Glass, *J. Neurosci*. 2005, 25:2637-46). Mit fortschreitendem Alter verringert sich die Zahl der Stammzellen im Gehirn, und damit verringert sich auch der Anti-Krebs-Effekt. Die Wahrscheinlichkeit, dass sich ein Gehirntumor bildet, ist im Alter erhöht; die Verminderung der Zahl der Stammzellen könnte dazu beitragen. Gerd Kempermann studiert die Eigenschaften und das Verhalten neuronaler Stammzellen. Seine Arbeitsgruppe konnte in Mäusen zeigen, dass regelmäßige körperliche Aktivität den normalen, altersbedingten Verlust der Stammzellen im Hippocampus verhindern kann. Allerdings ging die Erhöhung der Stammzellaktivität nicht mit einer erhöhten Bildung von Nervenzellen einher, obwohl das Potential für adulte Neurogenese gesteigert war.

Fritz Rathjen analysiert, wie neuronale Aktivität die Bildung der Kontaktstellen zwischen den Nervenzellen (Synapsen) beeinflusst. Die Arbeitsgruppe untersuchte systematisch, welche Proteine sich durch neuronale Aktivität verändern und fanden CALEB, ein transmembranes Protein, das eine EGF-Domäne enthält. Neuronale Aktivität fördert eine Umwandlung von CALEB und führt dazu, dass die EGF-Domäne im Protein exponiert wird. Mäuse, die wegen einer Mutation im CALEB-Gen das entsprechende Protein nicht synthetisieren können, zeigen während der Bildung von Synapsen bestimmte funktionelle und elektrophysiologische Veränderungen. Die Anzahl und die Gestalt der Synapsen verändern sich aber nicht (R. Juttner et al., 2005, *Neuron 46*, 133-45).

Der Tastsinn beruht nicht nur auf einer einzelnen Wahrnehmung, sondern erlaubt die Empfindung von Textur, Temperatur und Schmerz. Definierte Nervenzellen in den sensorischen Ganglien innervieren die Haut und leiten Informationen über Textur, Temperatur und Schmerz an das Zentralnervensystem weiter, indem sie diese ins Rückenmark projizieren. Die Neuronen im Rückenmark verarbeiten diese sensorischen Informationen und leiten sie weiter in das Gehirn. Sensorische Nervenzellen, die leichte Berührungen der Haut erkennen, unterscheiden sich von denen, die schmerzvolle Hitzereize erkennen. Bisher konnten sie aber aufgrund ihrer Zellgestalt oder auf molekularer Ebene nicht unterschieden werden. Das Gebiet der Schmerzforschung entwickelt sich zurzeit sehr rasch, da sehr viele Moleküle identifiziert wurden, die für die normale Schmerzwahrnehmung wichtig sind und die Schmerz-Überempfindlichkeit bei Krankheiten regulieren. Ein Übersichtsartikel von Gary Lewin fasste diese Fortschritte kürzlich zusammen (Lewin et al., 2004 *Curr. Opin. Neurobiol.* 14, 443-9). In Zusammen-

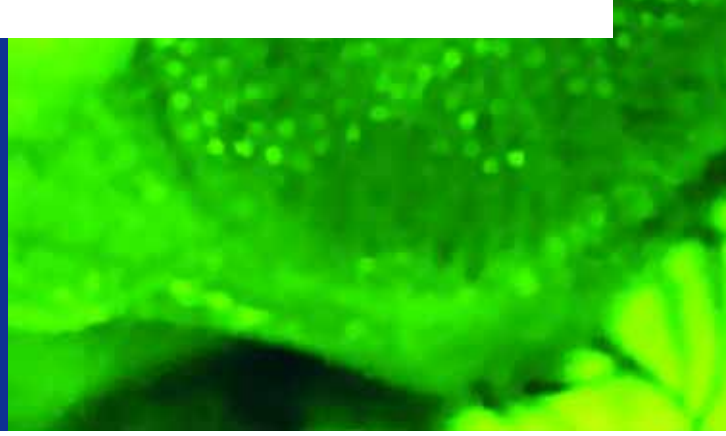
Thomas Müller in Carmen Birchmeier's group identified the function of a new transcription factor of the bHLH family, Olig3, in the development of particular neuron types in the dorsal spinal cord. These neurons function to transmit proprioceptive information, which they receive from sensory neurons in the periphery. This work was done in collaboration with Mathias Treier at the EMBL in Heidelberg (Müller et al. 2005 *Genes Dev.* 19, 733-43). Other factors that control the development of the dorsal horn have been identified by Stefan Britsch and are currently analyzed using mutant mice.

Inés Ibañez-Tallon joined the Neuroscience research program to establish her own group during the reported period. In her post-doctoral work done at the laboratory of Nat Heinz at the Rockefeller University in New York, Inés Ibañez-Tallon had found that toxins that are tethered to the membrane by a GPI anchor can inhibit ion channels and neurotransmitter receptors. She now plans to use such toxins to silence transmission at particular synapses or in particular neural circuits in transgenic animals. A new research network funded by the German Science Foundation (DFG) was established in Berlin, the Sonderforschungsbereich (SFB) "Developmental Disturbances of the Nervous System". Ten MDC scientists of the Neuroscience department participate in seven projects in this SFB and the deputy speaker of the SFB is Carmen Birchmeier. Other activities included a meeting of the faculty and students of the Neuroscience Department in 2005 at Döllnsee in Brandenburg, which facilitated presentations and discussions of ongoing research as well as fostered interactions in a relaxed and informal environment. This department meeting alternates with the Berlin Neuroscience Forum that is attended by neuroscientists from all the neuroscience institutes in Berlin. The neuroscience seminar series that is organized jointly by all neuroscience faculty members continues to run successfully and has attracted many renowned scientists to the MDC.

arbeit mit Frederique Scamps in Marseille zeigte die Arbeitsgruppe von Gary Lewin kürzlich, dass sensorische Nervenzellen mit Rossetten-Gestalt einem bestimmten Typ von Mechanorezeptoren (D-hair Mechanorezeptoren) entsprechen, die bisher nur durch elektrophysiologische Methoden identifiziert werden konnten (Dubreuil et al. 2004 *J. Neurosci.* 24, 8480-8484). Alistair Garratt konnte zeigen, dass Mäuse mit Mutationen im c-kit Gen, das für einen Tyrosinekinase-Rezeptor kodiert, Schmerz nicht normal empfinden können. Er untersucht nun mit Gary Lewin die elektrophysiologischen Veränderungen in den sensorischen Nervenzellen.

Thomas Müller in der Arbeitgruppe von Carmen Birchmeier definierte die Funktion eines neuen Transkriptionsfaktors der bHLH Familie, Olig3, während der Entwicklung des Rückenmarks. Wenn Olig3 fehlt, können bestimmte Nervenzellen, die Information über die räumliche Position des Körpers empfangen und weitergeben, nicht gebildet werden. Diese Arbeit wurde zusammen mit Mathias Treier am EMBL in Heidelberg durchgeführt (Müller et al. 2005 *Genes Dev.* 19, 733-43). Auch Stefan Britsch hat Gene identifiziert, die für die Bildung von Nervenzellen im Rückenmark verantwortlich sind. Er untersucht, inwieweit die Informationsverarbeitung und -weiterleitung sensorischer Reize beeinträchtigt ist, wenn solche Gene mutiert sind.

Das Forschungsprogramm Neurowissenschaften konnte in diesem Berichtszeitraum eine neue Gruppenleiterin, Inés Ibañez-Tallon, willkommen heissen. Während ihrer Post-doc-Zeit im Labor von Nathaniel Heintz (Rockefeller University, New York) hatte sie gefunden, dass bestimmte Toxine, die durch einen GPI-Anker an der Zelloberfläche befestigt sind, Ionenkanäle und Neurotransmitter-Rezeptoren inhibieren. Sie plant solche Toxine zu nutzen, um die Funktion bestimmter Nervenzellen oder von Nerven-Schaltkreisen in transgenen Mäusen zu untersuchen. Außerdem wurde in Berlin ein neuer Sonderforschungsbereich „Entwicklungsstörungen im Nervensystem" (SFB 655) eingerichtet, an dem zehn Wissenschaftler des MDC in sieben Projekten beteiligt sind. Zu den weiteren Aktivitäten gehörte ein gemeinsames Symposium aller Neurowissenschaftler des MDC, das 2005 in Döllnsee, Region Brandenburg, durchgeführt wurde und als Forum für die Diskussion neuester wissenschaftlicher Ergebnisse und zur Planung weiterer Zusammenarbeiten genutzt wurde. Der Koordinationsbereich führte im Berichtszeitraum ein gemeinsames Vortragsprogramm durch, das für viele Wissenschaftler und Studenten sowohl im Neuro- als auch aus anderen Bereichen des MDC attraktiv war, zumal namhafte Wissenschaftler in diesem Programm Seminare gaben.



Mouse Genetics – Tools for the Functional Analysis of Genes that are Important for Development and Regeneration

Carmen Birchmeier-Kohler

We are using mice as model organisms for the functional analysis of genes important for embryonic development and tissue maintenance. Tools for molecular genetics are well established in mice. Homologous recombination and embryonic stem cell technology make it possible to introduce targeted deletions or insertions into the mouse genome. A further development of the technique, the Cre-LoxP technology, allows for the introduction of subtle alterations, like point mutations or conditional mutations, that are restricted to a particular cell lineage.

Development of the spinal cord

Thomas Müller, Henning Brohmann, Hagen Wende, Dietmar Zechner, Mathias Gierl, Hendrik Wildner, Dominique Bröhl

The dorsal horn of the spinal cord is the first central relay station for somatosensory perception. Interneurons and projection neurons in the dorsal horn integrate incoming sensory information and transmit this information to higher brain centers. The assembly of these complex neuronal circuits depends on the generation of functionally distinct types of dorsal horn neurons. Physiological studies have defined many distinct populations of dorsal horn neurons, which (i) process sensory information associated with touch, pain, and heat perceptions, (ii) modulate reflex-specific motoneuron output, and (iii) relay afferent sensory information to the brainstem and the thalamus. Neurons with different physiological properties are segregated in distinct laminae of the dorsal horn. The cascade of events that specifies the development of these different neuronal subtypes is unclear and, thus, we are analyzing this process using mouse genetics.

The dorsal part of the spinal cord gives rise to neurons that process and relay sensory information, whereas the ventral part generates motoneurons and interneurons that coordinate motor output. During an early developmental phase called the first neurogenic wave (E10.5 in the mouse), distinct types of neurons arise at stereotyped positions along the dorso-ventral axis in the developing spinal cord. These neurons settle in deep layers of the dorsal horn or in the ventral spinal cord. During a later phase known as the second neurogenic wave (E12.5 in the mouse), most neurons of the dorsal horn are born. At the time of birth of these late neurons, only two subtypes can be distinguished that settle in the upper layers of the dorsal horn. We systematically investigated the genes expressed in the dorsal horn by affymetrix chip analyses and currently analyze the function of a number of these genes in dorsal horn development.

The role of Olig3 in spinal cord development

Thomas Müller, Hendrik Wildner

Class A and B neurons emerge in the dorsal and ventral alar plate, differ in their dependence on roof plate signals for specification, and settle in the deep and superficial dorsal horn, respectively. We showed that the basic helix-loop-helix (bHLH) gene Olig3 is expressed in progenitor cells that generate class A (dI1-dI3) neurons and that Olig3 is an important factor in the development of these neuronal cell types. In Olig3 mutant mice, the development of class A neurons is impaired: dI1 neurons are generated in reduced numbers whereas dI2 and dI3 neurons are misspecified and assume the identity of class B neurons. Conversely, Olig3 represses the emergence of class B neurons in the chick spinal cord. We conclude that Olig3 expression distinguishes the two major classes of progenitors in the dorsal spinal cord and determines the distinct specification program of class A neurons. This project was done in collaboration with the laboratory of Mathias Treier at the EMBL in Heidelberg.

beta-Catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the spinal cord

Dietmar Zechner, Thomas Müller

beta-Catenin is an essential component of the canonical Wnt signaling system that controls decisive steps in development. We employed two conditional beta-catenin mutant alleles to alter beta-catenin signaling in the central nervous system of mice: one allele to ablate beta-catenin and the second allele to express a constitutively active beta-catenin. The tissue mass

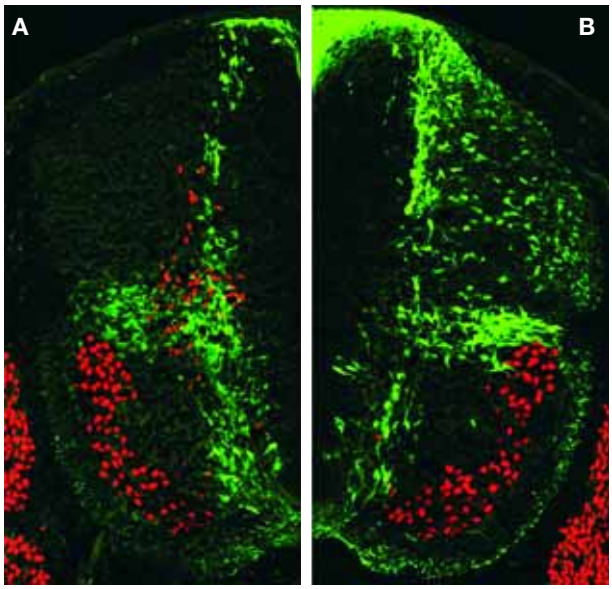
of the spinal cord and brain is reduced after ablation of beta-catenin, and the neuronal precursor population is not maintained. In contrast, the spinal cord and brain of mice that express activated beta-catenin is much enlarged in mass, and the neuronal precursor population is increased in size. beta-Catenin signals are thus essential for the maintenance of proliferation of neuronal progenitors, controlling the size of the progenitor pool and impinging on the decision of neuronal progenitors to proliferate or to differentiate.

The Role of the Met in liver regeneration

Malgorzata Borowiak, Michael Strehle

Genetic analysis in mice has demonstrated a crucial role of the Met tyrosine kinase receptor and its ligand, hepatocyte growth factor/scatter factor (HGF/SF), in the development of the liver, muscle, and placenta. We used conditional mutagenesis in mice to analyze the function of Met during liver regeneration, using the Mx-cre transgene to introduce the mutation in the adult. After partial hepatectomy in mice carrying the Mx-cre-induced Met mutation, regeneration of the liver is impaired. Comparison of signal transduction pathways in control and mutant livers indicates that Met and other signaling receptors cooperate to fully activate particular signaling molecules, for instance, the protein kinase Akt. However, activation of the Erk1/2 kinase during liver regeneration depends exclusively on Met. Signaling crosstalk is thus an important aspect of the regulation of liver regeneration. Analysis of cell cycle progression of hepatocytes in conditional Met mutant mice indicates a defective exit from quiescence and diminished entry into S phase. Impaired liver regeneration is accompanied by compensatory physiological responses that include prolonged up-regulation of HGF/SF and IL-6 in peripheral blood. Our data demonstrate that the HGF/SF/Met signaling system is essential not only during liver development but also for the regeneration of the organ in the adult. This project was done in collaboration with Torsten Wüstefeld and Christian Trautwein from the Medizinische Hochschule in Hannover.

Figure 1. The basic helix-loop-helix transcription factor Olig3 promotes the specification of class A dorsal neurons in the developing spinal cord. Shown here are two immunofluorescence images of spinal cords from E12.5 mouse embryos heterozygous **(A)** or homozygous **(B)** for an *Olig3^{GlLacZ}* allele in which the *Olig3* coding sequence was replaced by a *GFP-IRES-LacZ* cassette. The sections were analyzed using antibodies directed against GFP (green) and the class A dI3 neuron subtype marker, Isl1 (red). The homozygous mutant shows the ectopic accumulation of GFP+ neurons to lateral positions in the dorsal spinal cord.



CXCR4 and Gab1 cooperate to control the development of migrating muscle progenitor cells

Long-range migrating progenitor cells generate hypaxial muscle, for instance the muscle of the limbs, hypoglossal cord, and diaphragm. We have previously shown that their migration requires signals of the tyrosine kinase receptor Met and its signal transducer Gab1. We recently found that migrating muscle progenitors express the chemokine receptor CXCR4. The corresponding ligand, SDF1, is expressed along the routes and at the targets of the migratory cells. Ectopic application of SDF1 in the chick limb attracts muscle progenitor cells. In CXCR4 mutant mice, the numbers of muscle progenitors that colonize the anlage of the tongue and the dorsal limb were reduced. Changes in the distribution of the muscle progenitor cells were accompanied by increased apoptosis, indicating that CXCR4 signals provide not only attractive cues but also control survival. Gab1 encodes an adaptor protein that transduces signals elicited by tyrosine kinase receptors, for instance the c-Met receptor, and plays a role in the migration of muscle progenitor cells. We found that CXCR4 and Gab1 interact genetically. For instance, muscle progenitors do not reach the anlage of the tongue in CXCR4;Gab1 double mutants- this target is colonized in either of the single mutants. Our analysis reveals a role of SDF1/CXCR4 signaling in the development of migrating muscle progenitors and shows that a threshold number of progenitor cells is required to generate muscle of appropriate size.

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Muller T, Anlag K, Wildner H, Britsch S, Treier M, Birchmeier C. (2005) The bHLH factor Olig3 coordinates the specification of dorsal neurons in the spinal cord. Genes Dev. 19, 733-43.

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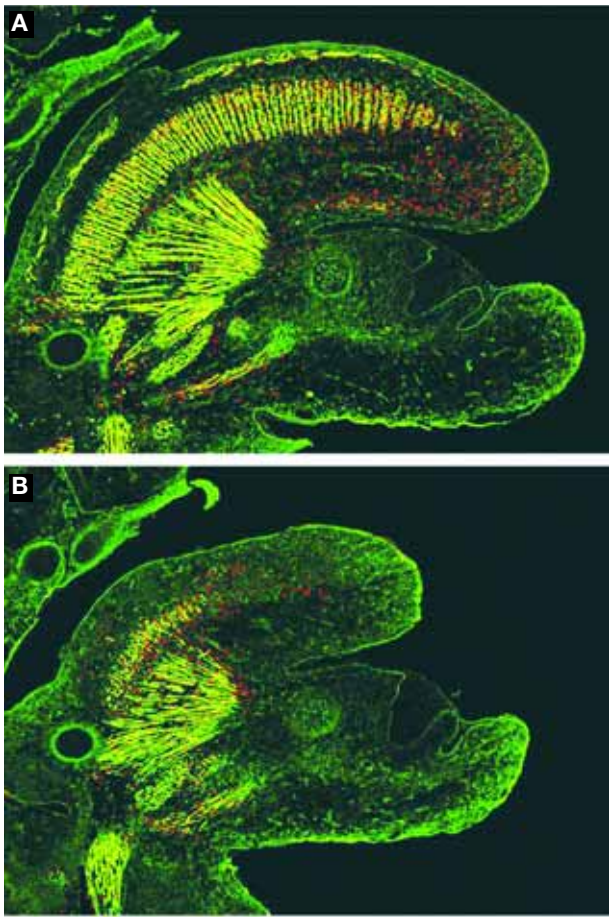

Figure 2. Differentiated muscles in the tongue of control **(A)** and CXCR4/Gab1 double mutant **(B)** mice were visualized by immunohistological staining using myosin (green) and myoD (red) specific antibodies. Two progenitor cell populations contribute to tongue muscles, long-range migrating muscle progenitor cells that derive from somites and muscle progenitor cells that originate from the head mesoderm. In CXCR4/Gab1 double mutant mice, long-range migrating muscle progenitor cells do not reach the anlage of the tongue. As a consequence the tongue muscle groups of somitic origin are lacking in such mutants.

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Molecular Control of Spinal Cord and Peripheral Nervous System Development

Stefan Britsch
(Helmholtz Fellow)

Our group is interested in the identification and functional characterization of novel genes involved in the development of the dorsal spinal cord and peripheral nervous system.

Genomic and functional analysis of dorsal spinal cord development

The dorsal spinal cord processes information from nociceptive, mechanosensory, and proprioceptive primary sensory neurons and relays it to higher brain centers. The cellular basis of these functions resides in a large number of interneurons that are located within distinct laminae of the dorsal spinal cord. The molecular characteristics of these interneurons, as well as the mechanisms underlying their development, are incompletely defined. In order to identify novel candidate genes with putative functions in the development of the dorsal spinal cord, we have performed global gene expression analyses with high-density oligonucleotide microarrays. By comparison of expression profiles of ventral and dorsal spinal cords, we identified genes that are enriched in the dorsal neural tube. Included among the differentially expressed genes are those with known functions in spinal cord development, as well as other genes with as yet unknown functions. Within this group of genes, we have identified the homeodomain transcription factor, Gbx1, and two C2H2 zincfinger transcription factors, Bcl11a and Bcl11b.

Recent work from our group has demonstrated that Gbx1 is expressed specifically in a subset of Lbx1$^+$ (class B) neurons in the dorsal spinal horn. Expression of Gbx1 in the dorsal spinal cord depends on Lbx1 function. Immunohistological analyses revealed that Gbx1 identifies a distinct population of late-born, Lhx1/5$^+$, Pax2$^+$ neurons. In the perinatal period, Gbx1 marks a subpopulation of GABAergic neurons. The expression of Gbx1 suggests that it controls the development of a specific subset of GABAergic neurons in the dorsal horn of the spinal cord. We have generated loss-of-function mutations of the Gbx1 gene in mice for further analysis of its role in the developing spinal cord.

Bcl11a and b are closely related C2H2 zincfinger transcription factors, which have recently been shown to be essential for lymphocyte development. Both genes are also expressed in the embryonic brain, spinal cord and peripheral nervous system. To analyze their functions during nervous system development, we have generated CNS-specific conditional mouse mutants for the Bcl11a and b genes (collaboration with Neal Copeland, NCI Frederick). Conditional mutant animals die after birth, indicating that both genes serve critical functions during nervous system development. Our preliminary phenotype analysis of the Bcl11a mutants demonstrates that the gene is critical for neuronal differentiation and connectivity within the dorsal spinal horn.

Functional analysis of the Prospero-related homeodomain transcription factor Prox2 during development of viscerosensory neurons

In vertebrates, peripheral sensory neurons derive either from placodal or from neural crest cells. Placodes are focal regions of thickened ectoderm in the vertebrate head, which give rise to both neuronal and non-neuronal cells. Cranial placodes include a series of three epibranchial placodes which generate viscerosensory neurons. Such neurons relay taste stimuli to the brainstem and participate in neuronal cricuits that are critical for the central control of respiration and heart function. The molecular mechanisms, however, that control induction and differentiation of sensory neurons from placodal ectodermal cells are still incompletely understood. We have shown that the novel murine *Prospero*-related homeodomain transcription factor Prox2 is exclusively expressed in a subgroup of placode-derived viscerosensory neurons. Members of the *Prospero*-family have been previously demonstrated to control cell-fate decisions and neuronal differentiation in the developing nervous system. We have therefore generated mice with a targeted deletion of the Prox2 gene to further analyze the functions of this gene during embryonic development of viscerosensory neurons.

Selected Publications

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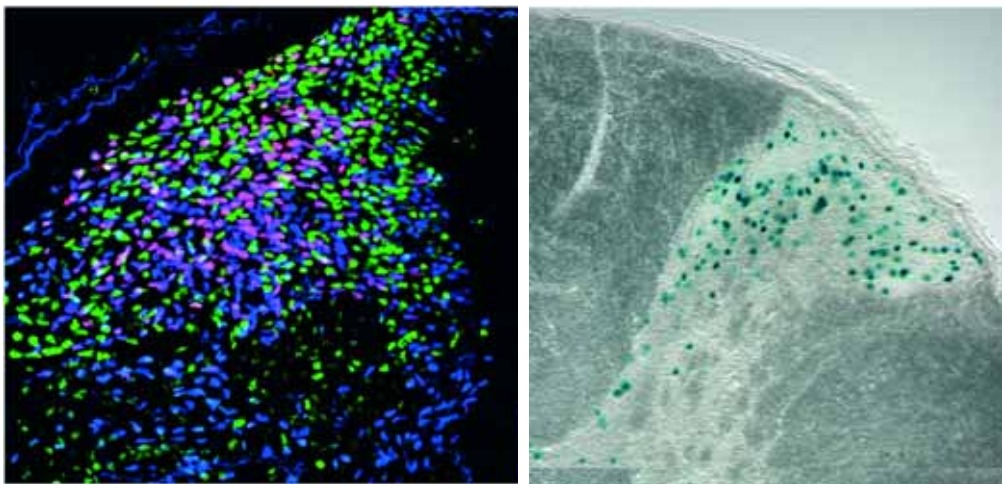
Müller T, Anlag K, Wildner H, Britsch S, Treier M, Birchmeier C (2005) The bHLH factor Olig3 coordinates the specification of dorsal neurons in the spinal cord. *Genes Dev* 19: 733-743.



Expression of the homeodomain transcription factor Gbx1 in the dorsal spinal cord. **Left:** Immunohistological detection of Gbx1 positive neurons (magenta) in the embryonic spinal cord (E12.5). Gbx1 positive neurons comprise a specific subpopulation of late-born dorsal neurons, that co-express Lhx1/5 (blue), but not the marker Lmx1b (green). **Right:** Gbx1 expression in the adult dorsal spinal horn, visualized by a lacZ reporter knocked into the genomic locus of the corresponding gene.

Structure of the Group

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Defining Novel Molecular Components of the Pain Pathway

Alistair N. Garratt
(Helmholtz Fellow)

The sense of pain is an essential survival mechanism, alerting the animal to environmental dangers and triggering rapid avoidance measures to prevent damage to the organism. The sensitivity of pain-sensing circuits can also be modified, for example nerve endings within damaged/inflamed tissue become hyperalgesic, a process which is designed to prevent further damage to the injury site. In the clinical setting, inappropriate and persistent activation of pain circuits underlies the development of chronic and neuropathic pain, debilitating conditions that are generally refractory to current modes of treatment. We employ classical and conditional (tissue-specific) inactivation of particular genes in the mouse in order to gain understanding into the molecular bases of pain processing and the development of pain-sensing circuits. We are currently focusing on the function of c-Kit, and the murine homologues of the *Teashirt* gene, which were identified in a screen of mRNA expression in the *substantia gelatinosa* of the spinal cord, an area of particular importance for the reception of pain stimuli.

Function of the receptor tyrosine kinase c-kit in pain sensing

Mutations in the genes encoding c-Kit, and its ligand, mast cell growth factor/stem cell factor/Kit ligand, were identified initially as being causal for dominantly-inherited pigmentation abnormalities in the mouse. *C-Kit* encodes a receptor tyrosine kinase related to the receptors for colony-stimulating factor and platelet-derived growth factor. The ligand, stem cell factor (SCF), is a transmembrane protein, which can also be proteolytically cleaved and function as a paracrine growth factor. This signaling system is essential for the development of three lineages, primordial germ cells, melanocytes, and hematopoietic stem cells. Both c-Kit and its ligand are also widely expressed in other tissues. In particular, c-Kit is expressed in certain sensory fibres, including a subset of pain-sensing neurons with axon termini located in the upper layers of the skin, where the ligand, SCF, is expressed. Studies on the function of the c kit/SCF signaling system in the adult mouse have been precluded by the perinatal lethality of null mutant mice. This phenotype can now be rescued through transgenic overexpression of erythropoietin. We are currently characterising the sensitivity of adult homozygous *c-Kit* mutant mice to mechanical and pain stimuli, using both in vivo (thermal and mechanical stimuli) and ex vivo approaches (single fibre recordings from skin-nerve preparations).

The three mammalian homologues of the Drosophila gene Teashirt

One group of genes expressed in the pain circuitry of the developing and adult mouse encodes the three homologues of the zinc-finger protein Teashirt, which was originally characterised to be essential for segmentation and cuticle development in the fruitfly. Using antibodies specific for the different Teashirt proteins, as well as in situ hybridisation, we are characterising the expression of these genes in dorsal root ganglia and spinal cord, as well as other tissues in the mouse. We are generating strains of mice carrying inactivating and conditional mutations in the *Teashirt* genes. Through the use of green fluorescent protein and beta-galactosidase as markers, we will track the fate of mutant cells and analyse the phenotypic consequences on nervous system development. We will also employ conditional mutagenesis to analyse the function of the *Teashirt* genes in pain-sensing circuits of adult mice.

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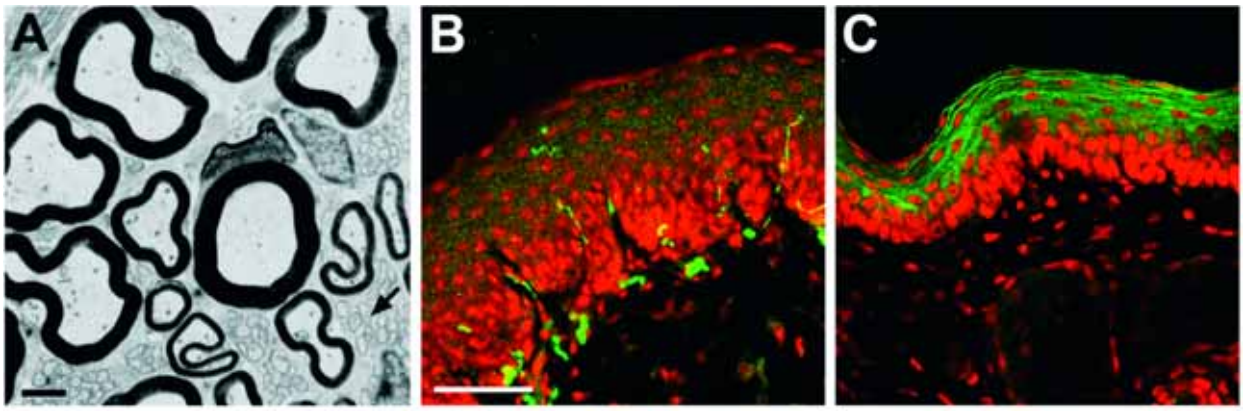

Axons of sensory neurons course through peripheral tissues to their target sites. **A**, electron micrograph of a peripheral nerve, showing myelinated axons ensheathed by dark electron-dense myelin sheaths (fast conduction of stimuli), and small diameter slowly conducting unmyelinated fibres (arrow), which innervate cutaneous tissues and process noxious pain stimuli. Immunohistochemistry of sections through the skin to detect calcitonin gene-related peptide, expressed in small diameter sensory neurons terminating in the upper layers of the skin (**B**, green) where the ligand for the receptor tyrosine kinase c-Kit, SCF is located (**C**, green). Nuclei are shown in red. Bars, A, 1 μm; B, 50 μm.

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Cellular Neurosciences

Helmut Kettenmann

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 new 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. 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 three topics: (1) the role of astrocytes in information processing (2) the response of microglial cells to brain injury and (3) the cellular properties of gliomas.

1. How do astrocytes communicate among each other?

From experiments in cell culture and from studies in the isolated retina it has become evident that astrocytes can communicate over large distances (< 0.5 mm) via calcium signalling in the form of waves. We have found conditions to elicit and record such Ca^{2+} waves in slices containing the corpus callosum. In this white matter tissue, the communication among the astrocytes is mediated by ATP release and activation of purinergic receptors. The calcium waves spread over a large distance involving more than a hundred cells and travels with a low speed of about 10 μm/s. In contrast, calcium wave propagation in the neocortex depends on functional astrocytic gap junctions but is still accompanied by ATP-release. In acute slices obtained from the neocortex of mice deficient for astrocytic expression of connexin43, the calcium wave did not propagate. In addition to calcium wave propagation in astrocytes, ATP-release was recorded as a calcium signal from "sniffer cells", a cell line expressing high-affinity purinergic receptors placed on the surface of the slice. The astrocyte calcium wave in the neocortex was accompanied by calcium signals in the "sniffer cell" population. In the connexin43-deficient mice, we recorded calcium signals from sniffer cells also in the absence of an astrocytic calcium wave. Our findings indicate that astrocytes propagate calcium signals by two separate mechanisms depending on the brain region and that ATP release can propagate within the neocortex independent from calcium waves.

2. What are the physiological features of microglial cells in brain tissue?

Microglial cells are the major immunocompetent cells in the brain and express many features of monocytes. This includes signalling cascades well described in the immune system involving chemokines and cytokines and their receptor systems. In this project, we addressed the question whether microglia would also express receptors to sense neuronal activity. We have recently developed an *in situ* model which allows 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 GABAB 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 which are important signalling molecules for astrocyte activity. Chronic dopamine receptor stimulation enhanced migratory activity and attenuated the lipopolysaccharide (LPS)-induced NO release similar as by stimulation of adrenergic receptors. While, however, noradrenaline attenuated the LPS induced release of TNF alpha and IL-6, dopamine was ineffective in modulating this response. Thus microglia express dopamine receptors which are distinct from adrenergic receptors In conclusion, microglial cells express a variety of the classical neurotransmitter receptors. These findings support the hypothesis that microglial cells are less prone to activation when they sense normal neural activity.

3. Are microglial cells important for neuronal reorganization after injury?

A candidate for signalling neuronal injury to microglial cells is the CC chemokine CCL21, since damaged neurons express CCL21. Investigating microglia in acute slices and in culture, we demonstrate that CCL21 triggers a Cl^- conductance increase. Moreover, CCL21 triggers a chemotactic response, which is sensitive to Cl- channel blockers. Both types of responses are mediated by activation of CXCR3 and not CCR7 receptors indicating that in brain, CCL21 acts via a different receptor system than in lymphoid organs. We have now tested

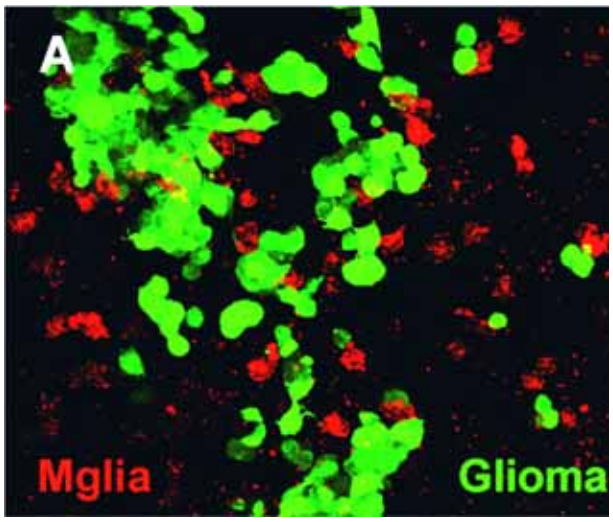

Figure 1. Immune cell of the brain (microglia) increase the invasiveness of brain tumours (glioma).
The micrograph shows Microglia (Mglia, in red) accumulating in a glioma (green).

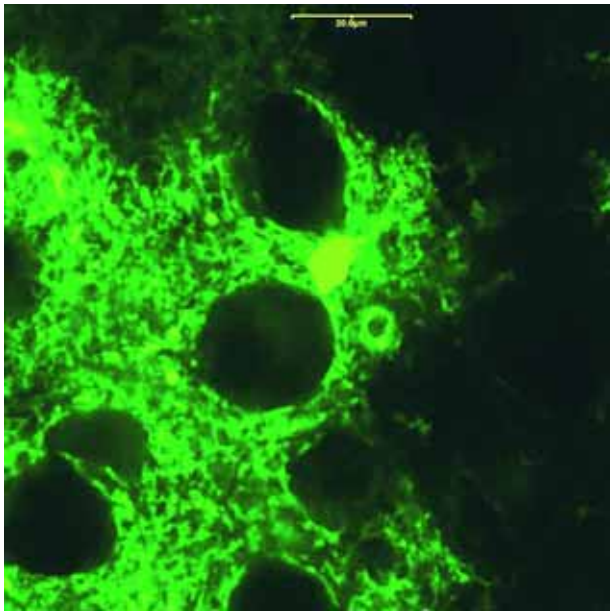

Figure 2. Overlay of five pictures of a GFAP-EGFP brainstem slice in the region of the MNTB. A GFAP expressing glial cell (a) positioned between two principal neurons (*) and in contact with a capillary.

the impact of CXCR3 signalling on cellular responses after entorhinal cortex lesion. In wild type mice, microglia migrate within the first 3 days after lesion into the zone of axonal degeneration, where 8 days after lesion denervated dendrites of interneurons are subsequently lost. In contrast, the recruitment of microglia was impaired in CXCR3 knockout mice and, strikingly, denervated distal dendrites were maintained in zones of axonal degeneration. No differences between wild type and knockout mice were observed following facial nerve axotomy, as a lesion model for assessing microglial proliferation. This shows that CXCR3 signalling is crucial in microglia recruitment, but not in proliferation, and this recruitment is an essential element for neuronal reorganization. This research is funded by a binational grant with Erik Boddeke, Groningen.

4. 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 are thought to originate from glial cells. These include astrocytomas, oligodendrogliomas, and the most malignant (and untreatable) brain tumor, the glioblastoma multiforme. We study the cellular properties of these tumor cells and compare them to normal glial cells with respect to their physiological properties and their abilities to proliferate and migrate. Currently, we addressed the question whether microglial cells influence tumor cell behaviour. In a slice culture, we injected a defined amount of tumor cells and quantified their migration within tissue. We found that microglial cell depletion from the slice slowed tumor invasion. Thus, the presence of microglial cells promotes the invasion of tumor cells. This research is funded by a binational grant with Bozena Kaminska, Warsaw.

5. Do stem cells influence glioma cells?

We recently observed that gliomas attract neural precursor cells from the germinal zone. These cells migrate over large distances and enwrap the tumor and they do not originate from the tumor proper as previously anticipated. 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. This has an interesting clinical correlation: glioblastoma occur in elderly patients but are virtually absent from young. Thus, stimulation of precursor cell activity could be of benefit for glioma patients.

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Brain Energy Metabolism

Susanne Arnold
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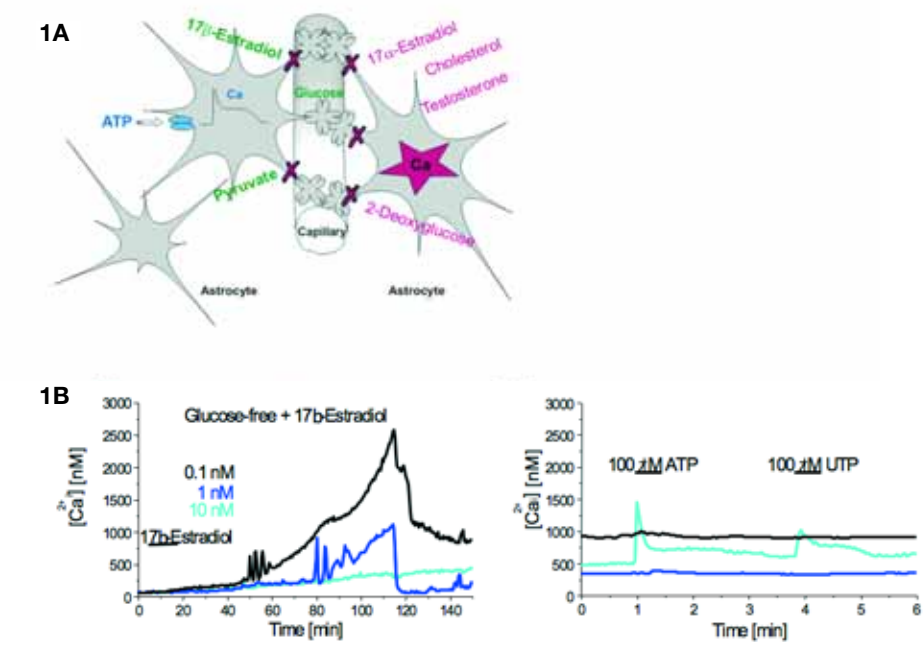
The brain is the organ with the highest energy demand in mammalian organisms. Neurons and astrocytes, two different brain cell types, are structurally, functionally, and metabolically tightly coupled with astrocytes playing a central role in regulation of cerebral energy metabolism in dependence on neuronal activity. Glucose and oxygen are the two most important substrates that fulfil neuronal energy requirements.

We, therefore, investigated the effect of glucose deprivation on astrocytic function and intercellular communication and found that, under those conditions, astrocytes show an elevation of intracellular calcium equivalent to an impairment of astrocytic neurotransmitter signalling (figure 1). The hormone, 17β-estradiol, protects astrocytes from the impact of glucose deprivation on resting Ca^{2+} levels and on neurotransmitter-triggered Ca^{2+} signalling independently of the nuclear estrogen receptor.

Oxygen, as the other most important substrate for brain energy metabolism, exerts a direct effect on mitochondrial oxidative energy production: on the one hand, oxygen is the substrate of cytochrome c oxidase (COX), the terminal enzyme of the respiratory chain; on the other hand, oxygen deprivation leads to structural and functional changes of this enzyme. Mammalian COX is composed of three catalytic, mitochondrially-encoded and ten regulatory, nuclear-encoded subunits. The regulatory COX subunit IV plays an important role in adjusting energy production to energy requirements by binding ATP to the N-terminus of subunit IV and, thereby, causing an allosteric inhibition of COX activity at high energy level, i.e. high ATP/ADP ratio [Kadenbach et al., 2004]. It was found that this COX subunit is expressed in isoforms (IV-1 and IV-2). While isoform IV-1 is ubiquitously transcribed in all adult mammalian tissues including brain, isoform IV-2 showed so far high transcription levels only in the lung. Under conditions of oxygen deprivation mRNA transcription of COX IV-2 is induced in astrocytes (see figure 2B). So far, yeast has been the only organism known to express two isoforms (Va and Vb), homologous to the mammalian subunit IV, in dependence on oxygen concentration. Functional consequences of increased expression of COX IV-2 isoform are reflected in an abolition of allosteric inhibition of COX by ATP at high ATP/ADP levels (figure 2C). We conclude that hypoxia-induced structural changes of COX complex suppress the COX sensitivity to its allosteric regulator ATP and overrule the regulation of COX and mitochondrial energy production by the cellular energy level.

Figure 1. Effect of glucose deprivation on intracellular calcium concentration and neurotransmitter signaling of astrocytes.
A Schematic drawing: Glucose deprivation induces an increase of intracellular calcium in astrocytes which is not prevented by application of 17α-estradiol, cholesterol, testosterone, or 2-deoxyglucose, but by application of 17β-estradiol, or pyruvate.
B Increase of intracellular calcium (left graph, black trace) and impaired responsiveness of primary astrocytes from mouse brain cortices upon neurotransmitter application (right graph, black trace) due to glucose deprivation are prevented by ≥ 10 nM 17β-estradiol (cyan traces).



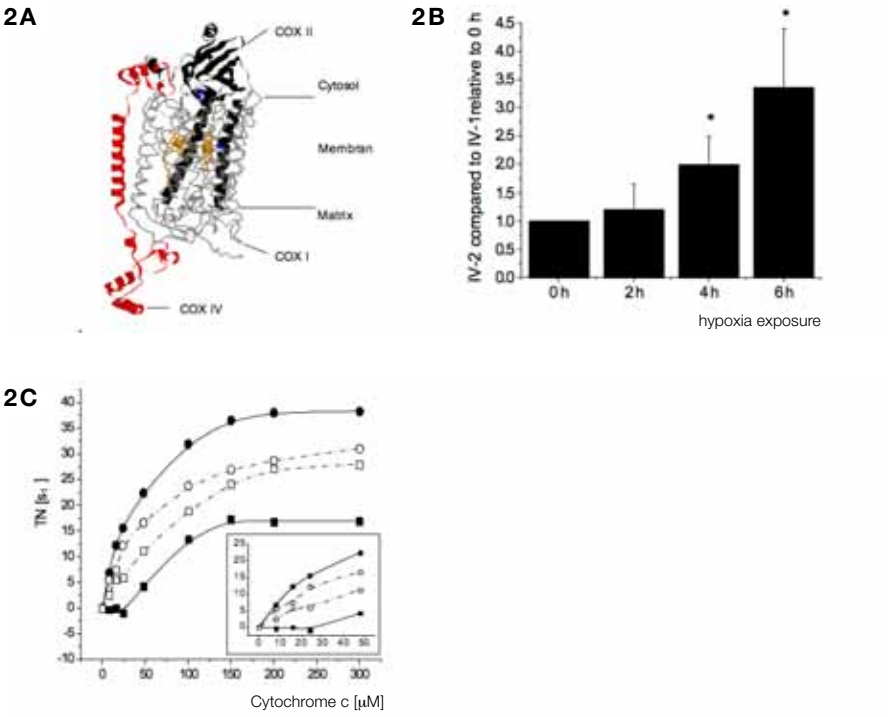


Figure 2. Effect of oxygen deprivation on structure and function of cytochrome c oxidase.
A Structure of the catalytic core of cytochrome c oxidase with catalytic subunit II (black) and regulatory subunit IV (red).
B Quantitative PCR using the TaqMan® system shows that mRNA transcription of COX IV-2 isoform in primary astrocytes from mouse brain is upregulated 3-fold after 6 hours of hypoxia.
C Polarographic measurement of cytochrome c oxidase activity (TN, consumed O2 [µM]/ total protein [mg] x s) of solubilized mitochondria from primary astrocytes in dependence on cytochrome c concentration after 6 h of normoxia (filled symbols) and after 6 h of hypoxia (open symbols) in the presence of ATP (squares) and ADP (circles). The allosteric inhibition of cytochrome c oxidase measured in the presence of ATP under normoxia (closed squares) is prevented after 6 h of hypoxic treatment (open squares).

Furthermore, we are developing molecular fluorescent biosensors for studying metabolic substances and parameters of intracellular signalling. A cytosolic and a mitochondrially targeted pH sensor were constructed by site-directed mutagenesis. Those pH sensors allow pH measurements in cytosol and mitochondria of cells in a semi-quantitative way and are used to investigate pH changes in astrocytes under physiological and pathological conditions.

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Selected Publications

Arnold, S. (2005). Estrogen suppressed the impact of hypoglycemia on astrocytic calcium levels and signaling independently of the nuclear estrogen receptor. Neurobiology of Disease, in press.

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Proteomics and Molecular Mechanisms of Neurodegenerative Disorders

Erich E. Wanker

A reductionist perspective has dominated the life sciences in the last century and, with regard to cell biology, has provided a wealth of knowledge about individual cellular components and their functions. Recently, however, it is increasingly clear that discrete biological functions can only rarely be attributed to discrete molecular entities in the cell. Instead, most cellular functions are carried out by complexes or modules of molecules, more specifically of proteins, that form in cells in certain spaces at certain times.

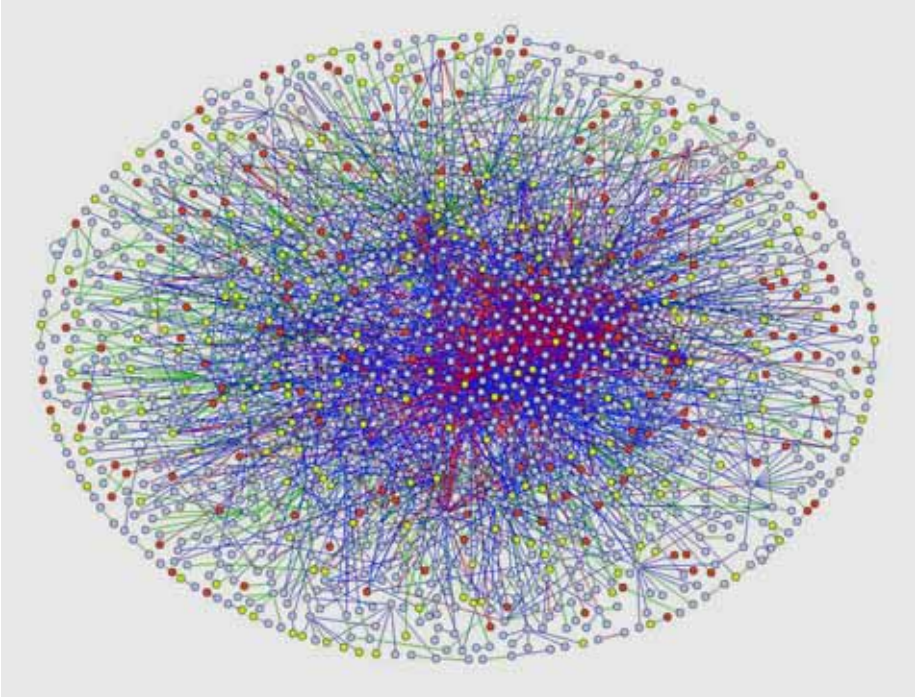
The main objective of our work is to understand the cell's functional organization and to link individual proteins to signalling cascades and disease processes. For the systematic identification and validation of protein-protein-interactions and the analysis of gene regulatory networks, we have established high-throughput technologies. Using an automated yeast two-hybrid system (Y2H) system we have, for example, generated the first human protein interaction map consisting of 3,186 interactions among 1,705 proteins. In addition, an interaction network for Huntington's disease was created, which allowed the identification of GIT1, a potential modifier of disease pathogenesis. These data are now available in a web-based searchable database for the scientific community and will serve as an important source of information for the study of the molecular organization of human cells and disease mechanisms. Closely linked to these goals is our work on the identification and functional characterization of small molecules that slow down aggregate formation in neurodegenerative diseases such as Huntington's and Alzheimer's disease. Lead compounds are currently investigated pharmacologically and tested for their activity in different transgenic disease models.

From interaction networks to disease modifiers

Using a combination of library and matrix Y2H screens, we have generated a protein-protein interaction network for Huntington's disease that contains 188 mostly novel interactions between 86 different proteins. As several lines of evidence indicate that aggregation of mutant huntingtin is linked to disease progression and the development of symptoms, we have searched for network proteins that can either enhance or slow down huntingtin aggregation. Utilizing a cell-based assay, we could identify GIT1, a G protein-coupled receptor kinase-interacting protein, that dramatically stimulates the formation of huntingtin aggregates. Mechanistic studies revealed that this effect is due to the recruitment of huntingtin to vesicles, which form in cells with a higher frequency when GIT1 is overexpressed. Coimmunoprecipitation experiments also showed that GIT1 and huntingtin interact in neurons, indicating that both proteins associate under physiological conditions. Moreover, immunohistochemical studies demonstrated that GIT1 is present in neuronal nuclear inclusions, a key feature of Huntington's disease, suggesting that loss of GIT1 function or mislocalization of the protein in neurons contributes to the pathogenesis. This hypothesis is also supported by biochemical studies indicating that full-length GIT1 is abnormally cleaved in patient brains but not in healthy individuals. Our results show that systematic interaction mapping studies combined with hypothesis driven approaches focusing on specific medically relevant questions can lead to the identification of potential disease modifiers.

A human protein interaction network

Besides focussing on proteins involved in disease processes such as Huntington's disease, we performed a systematic interaction mapping study to generate a human interactome network. This network is intended to serve as a resource for annotating the proteome and offers new insights into the molecular organization of human cells. By systematic interaction mating with our automated Y2H system, we tested about 25 million potential interaction pairs and identified 3,186 mostly novel interactions among 1,705 proteins. A significant fraction of these interactions was validated by independent pull-down assays and immunoprecipitations with an overall success rate of 65%. To enable a meaningful evaluation of the potential biological relevance of the interactions, a confidence scoring system was developed using experimental, topological and GO criteria. Interactions were ranked according to these criteria and a data set of 911 high confidence interactions among 401 different human proteins was defined. These interactions are promising starting points for further biological validation and the formulation of new hypotheses. All interactions identified by Y2H screening were stored in a web-based searchable database (www.mdc-berlin.de/neuroprot/database.htm) that permits queries for protein names, accession numbers, gene names and LocusLinkIDs. In order to demonstrate the usefulness of our interaction data for hypothesis driven biological research, we mapped the Y2H interactions to human gene regulatory pathways. Using bioinformatic tools, about 150 human proteins could be linked via high confidence interactions to the 22 different KEGG -Kyoto Encyclopedia of Genes and Genomes- pathways. This analysis also allowed the mapping of the proteins ANP32A and CRMP1 via Axin1 to the Wnt pathway. Using cell-based reporter assays, we then also demonstrated that these proteins can modulate Wnt signaling in vivo (collaboration with the research group of Walter Birchmeier). Together, these studies demonstrate that connecting proteins to signaling cascades via



Global View of the Y2H Interaction Map
In the Y2H interaction matrix screening, a total of 3,186 unique interactions between 1,705 different human proteins was identified. The complete network was drawn using the Pajek program package (A Program for Large Network Analysis by V. Batagelj and A. Mrvar at http://vlado.fmf.uni-lj.si/pub/networks/pajek/). The picture shows a giant connected network with 1,613 nodes and 3,131 links and 43 small networks with less than 6 nodes. The proteins and interactions are color-coded. We grouped the proteins in three broad categories using GO and OMIM criteria: disease proteins, according to OMIM morbidmap, NCBI (195, orange circles); uncharacterized proteins without GO and disease annotation (343, yellow circles) and known proteins with GO annotation (1167, light blue circles). Furthermore, we developed a scoring system to define interactions of high (HC, red lines), medium (MC, blue lines) and low confidence (LC, green lines).

Y2H interactions allows the identification of potential pathway modifiers that can be characterized further using *in vitro* and *in vivo* model systems.

Development of membrane-based human proteome arrays for systematic screening of protein-protein interactions

Besides the yeast two-hybrid system and affinity chromatography-based methods, protein arrays have become a valuable tool for high-throughput biology, because they allow the parallel analysis of thousands of proteins in a single experiment. We have developed a membrane-based proteome array technology that permits the screening of protein-protein interactions using crude bacterial cell extracts containing recombinant human proteins. This method makes the time consuming, expensive purification of the proteins unnecessary. We have applied our array technology in proof-of-principle experiments to identify novel partners for the human proteins CHIP, amphiphysin II and VCP/p97. Using this approach, many interaction pairs such as CHIP/caytaxin, amphiphysin II/DLP4 and VCP/AMFR were identified, and subsequently confirmed by pull-down, yeast two-hybrid and functional assays. Moreover, binding motifs in the amphiphysin II interacting proteins DLP4, XRCC4 and FBP were mapped using peptide arrays. These studies indicate that crude protein extracts can be utilized successfully for the identification of human protein-protein interactions and might also be applicable for systematic protein-drug and protein-DNA interaction screens.

The flavonoid D1 inhibits mutant huntingtin aggregation and reduces toxicity in disease model systems

Several sporadic and genetic diseases such as Huntington's, Parkinson's and Alzheimer's disease are caused by protein misfolding and the assembly of insoluble protein aggregates. A unified view of the molecular and cellular pathogenesis of these conditions has led to the search for small molecules that can slow, arrest, or revert disease progression. Using an automated filter retardation assay, we have identified about 300 chemical compounds that can reduce the formation of mutant huntingtin aggregation in cell-free and cell-based assays. Further analysis revealed that one of these compounds belongs to the group of flavonoids, which are natural substances with beneficial pharmacological activities. In addition to their antioxidative and anticarcinogenic properties, they have been shown to reduce memory loss and dementia in patients affected by neurodegenerative diseases. For this reason, we have analysed the effect of the flavonoid D1 and related substances on huntingtin, α-synuclein, and amyloid-β aggregation in different *in vitro* and *in vivo* model systems. We found that D1 is a potent inhibitor of polyQ-mediated huntingtin aggregation. Moreover, it prevents the fibrillogenesis of α-synuclein and amyloid-β in a concentration-dependent manner. Thus, D1

recognizes different amyloidogenic proteins and inhibits their misfolding and assembly into insoluble fibrillar structures. Using electron and atomic force microscopy, we observed that D1 stimulates the assembly of oligomeric particles with a diameter of 30-40 nm, while such structures were not detected in untreated control reactions. This indicates that D1 prevents the fibrillogenesis of amyloidogenic proteins by stimulating the production of off-pathway oligomers, which are formed early in the aggregation process.

As experimental evidence has been presented that soluble oligomers and/or protofibrils rather than fibrillar structures are the species that cause neurotoxicity in cells, we studied the effects of D1 treatment on aggregation and toxicity *in vivo*. In a yeast model system, overexpression of a mutant huntingtin fragment (htt72Q) causes toxicity as well as the assembly of insoluble protein aggregates. However, when the cells were treated with D1, formation of httQ72 aggregates and toxicity were significantly reduced. Similar results were obtained when transgenic HD flies expressing a pathogenic htt fragment with an expanded polyQ sequence in photoreceptors were treated with D1. This indicates that D1, a potent inhibitor of htt aggregation *in vitro*, is able to halt polyQ-induced neurodegeneration in a dose-dependent manner *in vivo*.

An arginine/lysine-rich motif in ataxin-3 is responsible for the interaction with VCP/p97, a modulator of aggregate formation and neurotoxicity

Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant neurodegenerative disorder caused by an elongated polyglutamine sequence in the protein ataxin-3 (Atx-3). We found that the AAA ATPase VCP (valosin-containing protein) interacts with Atx-3 *in vitro* and *in vivo*. Using peptide arrays and pull down experiments, we determined a VCP binding motif (VBM) in Atx-3. We demonstrated that a highly conserved potential nuclear localization signal consisting of four consecutive basic amino acids (RKRR) in Atx-3 serves as the recognition site for the molecular chaperone VCP. Previous studies have produced evidence that ATP binding and hydrolysis cause major conformational changes in the VCP hexamer and affect the interaction with adaptors. We investigated whether the association of VCP with Atx-3 is modulated by the addition of nucleotides such as ATP or ADP. Pull-down assays revealed that Atx-3 binds to VCP in the presence and absence of ATP while ADP completely prevented the protein interaction. This suggests that ADP changes the conformation of VCP and, thereby, reduces the binding affinity for Atx-3. Since VCP can act as a molecular chaperone and unfold protein substrates, we also tested whether it can influence polyQ-mediated Atx-3 aggregation *in vitro*. We found the VCP modulated the fibrillogenesis of Atx-3 in a concentration dependent manner, with low concentrations of VCP suppressing it. Strikingly, no such effect was observed with the polyQ-containing disease protein huntingtin, which does not contain an arginine/lysine-rich VCP interaction motif, demonstrating that the VCP/Atx-3 interaction is specific. *In vivo* studies with *Drosophila* models confirmed that VCP selectively modulates ataxin-3 aggregation and neurotoxicity. The chaperone VCP seems to utilize arginine/lysine motifs as recognition signals to alter the conformation of target proteins. Modulation of VCP levels might therefore influence the pathogenesis of spinocerebellar ataxia type 3, defining the VCP/ataxin-3 association as a potential target for therapeutic intervention in neurodegenerative disease.

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Developmental Neurobiology

Fritz G. Rathjen

Regulation of synapse formation by activity-dependent processes

The proper functioning of the nervous system requires the establishment of a precise and selective pattern of synaptic connectivity and an appropriate balance between excitatory and inhibitory synapses. The formation of these synapses in the central nervous system is a complex process and might be regulated by multiple molecules. Furthermore, during development and throughout adulthood, synapses are continuously structurally and functionally reconfigured, a process that is described by the term synaptic plasticity. Neuroscientists assume that this reconfiguration is dependent upon the electric activity of neurons. However, the mechanisms by which membrane depolarization, modulation of intracellular calcium levels, and action potential generation exert their influence on the number, distribution, and properties of synapses is currently more or less unknown. During embryonic development, neuronal activity might be initially spontaneous. Later on, however, activity is influenced by sensory experience. In particular, neuronal circuits appear to be very sensitive to sensory experience during specific early postnatal phases, termed critical periods, after which plasticity is then decreased. It is therefore a fascinating question as to how neuronal activity interacts with genetic instructions to form and modify synapses or circuits within the nervous system. Although synapse formation is primarily a hallmark of the embryonic and postnatal development of the brain, the understanding of these processes might also yield insight in learning and memory processes in the mature brain.

It is known that synaptic activity can induce a number of molecular changes including posttranslational modifications of synaptic proteins, regulation of gene activity or secretion of proteases. Nevertheless, our understanding of the function of these factors is at a very early stage and it is uncertain as to how these components restructure or direct the development of central synapses in vivo. We have therefore searched for neuronal cell surface components that are modulated by neuronal activity. Proteins that are regulated by activity are considered to be candidate components implicated in synaptic refinement and plasticity.

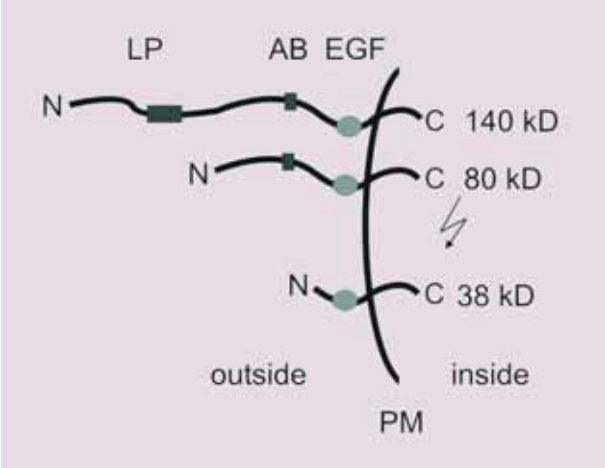
CALEB, an EGF-like protein processed by neuronal activity, is important for the function and development of synapses

One of our screens led to the identification of the transmembrane proteins CAR (coxsackie- and adenovirus receptor) and CALEB (chicken acidic leucine-rich EGF-like domain containing brain protein) that are regulated by neuronal activity. While the evidence implicating CAR in synaptogenesis is preliminary, there are compelling results that CALEB regulates pre-synaptic differentiation.

The characteristic feature of CALEB is an EGF-like domain close to its plasma membrane-spanning region that is related to TGFα or neuregulin-1. CALEB contains an acidic box that binds to tenascin-C and -R. CALEB becomes glycosylated by chondroitinsulfate chains at the N-terminus and is generated in at least two isoforms that differ in their cytoplasmic region. The EGF domain and the cytoplasmic stretch are highly conserved in vertebrates but proteins related to CALEB are not found in invertebrates.

Depolarization with elevated KCl or treatment with GluR agonists facilitate the conversion of CALEB at the plasma membrane resulting in a truncated transmembrane form with an exposed EGF domain. Different intermediate processing products are detectable in different cell types. While the reason for this conversion is currently not known it is conceivable that the EGF-like domain, which is likely to play a prominent role in the function of CALEB, becomes accessible upon processing for interactions with a yet unknown receptor. The converted form might therefore function as an activity-dependent juxtacrine signalling system and its regulation might provide a molecular basis for activity-dependent synaptic plasticity. CALEB is found throughout the nervous system and displays a developmentally regulated expression profile in many brain regions. For example, in the retina CALEB is predominantly localized in the optic fiber and inner plexiform layer, while in the cerebellum it is primarily associated with the Purkinje cells as well as the inner granular layer.

In the absence of CALEB, the number of synapses, their morphological characteristics, such as the number of docked vesicles at the active zone, as well as their postsynaptic properties, such as decay time constants of IPSCs, remained unchanged. However, CALEB gene inactivation alters the release features of synapses indicating that CALEB influences the function or the development of the presynapse. In acute slices of the colliculus superior of a CALEB-deficient mouse, GABAergic synapses displayed higher paired-pulse ratios, less depression during prolonged repetitive stimulation, a lower rate of spontaneous postsynaptic currents, and a lower neurotransmitter release probability. The molecular nature accounting for the CALEB dependency of the neurotransmitter release probability is not known. Interestingly, all measured effects of CALEB gene inactivation are confined to early stages of brain development. It is therefore conceivable that CALEB functions in the process of the assembly of



Activity-dependent conversion of CALEB.
Unprocessed CALEB is expressed at the plasma membrane that is converted in an activity-dependent manner, resulting in a transmembrane form composed of the EGF domain, the transmembrane region and the cytoplasmic segment. A prominent intermediate form in the chick retina is the 80 kDa component, which is further processed. Different intermediate processing products exists in different cell types. It cannot be completely excluded that the EGF domain is also released. AB – acidic box; LP – leucine, proline-rich region; PM – plasma membrane.

synapses. Absence of CALEB could then lead to a change in synaptic transmission.

The influence of neuronal activity on synapse development is most obvious under competitive situations. It is therefore of interest to study the activity-dependent regulation of CALEB under conditions where CALEB-positive and -negative neurons are simultaneously available in a network and to ask whether CALEB-deficient neurons are less successful in generating and maintaining synapses in comparison to wild-type synapses.

Additional projects

Other efforts of the group in the past granting period concentrated on the functional and molecular characterization of neurotractin, CAR, tenascin-R, intracellular trafficking proteins, and cGMP-mediated signalling in neurons. Of particular interest is CAR which resulted from the above mentioned screen on cell surface proteins modulated by neuronal activity. A CAR-deficient mouse that was generated by us to study the role of CAR in synaptogenesis dies very early in embryonic development long before synapses are generated. The reason for this lethality is due to malformations of the heart, a finding which was investigated in collaboration with Dietmar Vestweber and associates (MPI Münster, Germany). Our current studies investigate molecular interactions of CAR with extracellular matrix glycoproteins.

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Growth Factors and Regeneration

Gary Lewin

Sensory neurons of the dorsal root ganglia allow us detect stimuli to the body surface that lead directly to the sensations such as touch and pain. In my group, we are interested in the genes that allow these neurons to transduce different types of stimuli. Sensory neurons can, for example, detect changes in temperature of the skin in non-noxious (not painful) as well as the noxious range (painful heat or cold). They can also detect gentle movement of the skin as well as intense mechanical stimulation of the skin that is normally harmful. The nature of the transduction molecules involved together with the developmental events that lead to specification of the appropriate sensory neuron sub-types are actively investigated in my lab.

Molecular Basis of Mechanotransduction

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 detected 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 mammalian touch sensation. Some of these genes encode membrane ion channels of the Deg/EnaC superfamily that were proposed to open upon movement or displacement of the plasma membrane. We have previously shown that some mammalian Deg/EnaC channels belonging to the acid sensing ion channel sub-family (ASIC channels) are required for mice to properly discriminate touch stimuli. However, not all ASIC member channels appear to be essential. Interestingly, the expression of ASSDIC channels was recently shown by us to be regulated by neurotrophin availability. The mec genes in *C.elegans* have been proposed to work together in a mechanotransduction complex. Another 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 this gene and have created mouse knockout models to characterize the *in vivo* function of these genes. Our data indicate that the mammalian orthologs of mec-2 are also essential for normal mechanotransduction.

Mining the genome for sensory neuron markers

Sensory neurons in the dorsal root ganglia can be classified neurochemically or morphologically. However, probably the most important characteristic of sensory neurons is the modality of peripheral stimulus that they preferentially detect. Thus, some neurons respond to intense mechanical stimuli (nociceptors) and others respond only to the movement of the skin (Rapidly adapting mechanoreceptors). We have recently started to try and identify specific markers of these different physiological types by using genome wide microarray screens. We have taken advantage of mice with targeted deletions of neurotrophic factor genes that lose specific types of physiologically defined mechanoreceptors. For example, in mice null for the neurotrophic factor NT-4, one type of rapidly adapting mechanoreceptor, so-called D-hair receptors, are lost because they require specifically NT-4 for trophic support in the adult animal . We took advantage of this phenomenon to screen, using oligonucleotide microarrays, for genes that might only be expressed in D-hair receptors. One such gene found in this screen was a T type calcium channel that functions to enhance the mechanical sensitivity of this receptor type. Recently, in collaboration with a French lab, we have found that it is possible to isolate D-hair receptors in culture by their morphology and expression of high levels of T-type channels. We are extending this approach at the moment to find functionally important marker genes of other mechanoreceptor types including nociceptors. Using a microarray screen for genes in the mouse DRG that depend on nerve growth factor receptor, we have already identified several interesting genes that are being functionally characterized.

Hearing and touch

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 humans. Recently, we have started 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 that 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 now starting to measure pyschometric functions in hearing impaired patients and in unaffected controls in order to describe quantitatively potential differences in the perception of touch.

The Naked Mole Rat: a pain free mammal?

In collaboration with Dr Thomas Park at the University of Illinois, we have been characterizing the somatosensory system of the naked mole rat *(Heterocepahlus glabor)*. The

The naked mole rat a genome worth mining? These rodents have several unique features that make their genome of particular interest (see text). Picture courtesy of Thomas Park University of Illinois in Chicago.

naked mole rat is an unusual subterranean rodent in many respects. It is the only known poikilothermic mammal (i.e., cold blooded), lives in colonies with an insect-like social structure, and is also the longest-lived rodent species known (lifetime expectancy in excess of 25 years). Thomas Park noted previously that the sensory innervation of the skin in these mammals is devoid of two major neuropeptides, Substance P and Calcitonin gene related peptide. Since these two peptides are involved in nociception, we have made a detailed study of pain related behaviors in this species. Interestingly, although this animal has normal acute pain responses, it displays no hypersensitivity (so called hyperalgesia) to a variety of inflammatory and chemical stimuli. We suspect that at the heart of this specialized adaptation lies distinct gene variants encoding ion channels and associated channels that are required for the transduction of painful stimuli. We are at present starting to clone and characterize genes from the naked mole rat to address this issue.

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Neurodegeneration

Christiane Alexander

Our group is interested in the functional analysis of disease genes affecting the optic nerve. Optic neuropathies in infants and young adults, as well as glaucoma, are the main disorders we are concentrating on. Gene mapping strategies have identified several genes clearly involved in the disease pathology of optic nerve degeneration, like OPA1, mitochondrial NADH dehydrogenase, and Optineurin. We are focusing on the generation and analysis of animal models for those disorders and the biochemical characterization of the disease gene products.

OPA1 – the gene causing autosomal dominant optic atrophy

Mutations in the OPA1 gene cause a degeneration of the optic nerve in patients with autosomal dominant optic atrophy (adOA). adOA is the most prevalent hereditary optic neuropathy resulting in progressive loss of vision, visual field defects, bilateral temporal atrophy of the optic nerve, and colour vision defects with an onset within the first two decades of life. Histopathological post-mortem examination of donor eyes suggests that the fundamental pathology of adOA is a primary degeneration of retinal ganglion cells followed by ascending atrophy of the optic nerve. The symptoms of adOA are very similar to those observed in normal tension glaucoma, which can lead to problems in correctly diagnosing patients who present with mild visual disabilities.

By positional cloning, we were able to identify the *OPA1* gene, which spans about 100 kb of genomic sequence and is divided into 31 exons. Most mutations identified in adOA patients reside in the GTPase domain and in the exons coding for the very C-terminus of the OPA1 protein. A splicing hotspot involving exons 4, 4b, 5 and 5b was discovered recently, as well as, putative protein processing sites. Therefore, mRNA and protein processing may lead to up to 16 different isoforms of OPA1 in a human cell.

Expression analysis by Northern blot hybridisations revealed that *OPA1* was ubiquitously present in all tissues examined with the highest transcript level observed in retina, followed by brain, testis, heart, and skeletal muscle. Preliminary data from in-situ hybridization (ISH) experiments indicate predominant expression of the *OPA1* gene in the ganglion cell layer (GCL) which is consistent with the hypothesis of the pathophysiology of adOA.

The OPA1-GTPase is a Mitochondrial Protein

The OPA1 protein contains a classical N-terminal mitochondrial import signal which directs import of OPA1 into mitochondria. Furthermore, OPA1 shows homology to dynamin-related large GTPases that are conserved throughout evolution, like OPA1, DNM1 and Mitofusins. Recently, it was shown that these GTPases are involved in fusion and fission processes of membranes of mitochondria. Defects in these proteins lead to an abnormal distribution of mitochondria in the cell or deficits in mitochondrial function, e.g., respiration or apoptosis. While there are now ideas about the possible function of DNM1 and Mitofusins, the role of OPA1 for the cell and in the nervous system remains to be established.

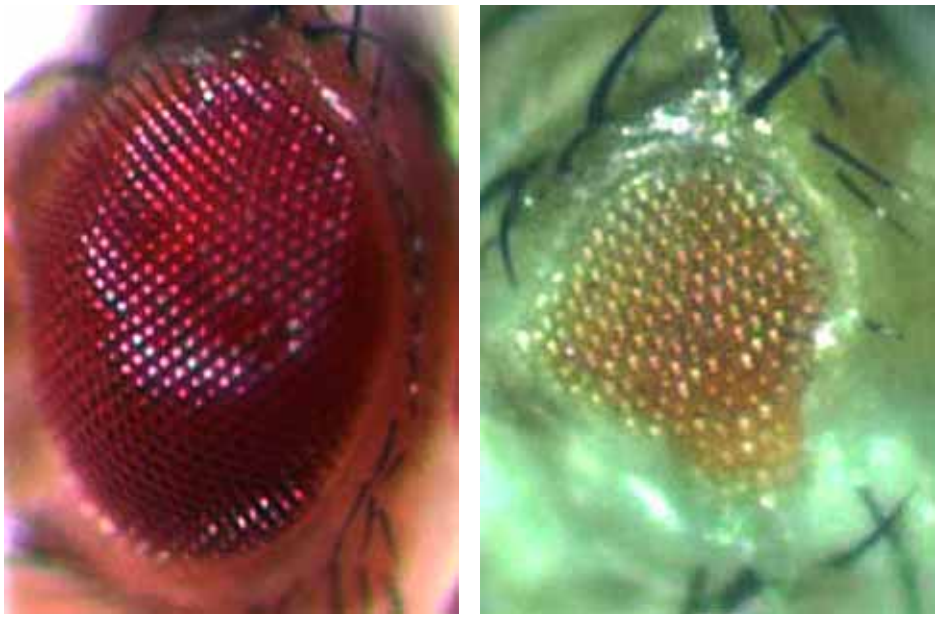
Manipulation of the OPA1 gene in animal models

We are gaining a first insight into OPA1 function by the generation of animal models such as genetically manipulated mice, flies and fish. We have observed that humans and all the animals studied in our lab die if OPA1 is completely absent. Therefore, we conclude that OPA1 is absolutely essential during embryonal development. The actual mitochondrial feature that is required at this stage and disturbed due to OPA absence is still under investigation.

In *Drosophila*, the genetic regulation of the visual system has been successfully studied in the past, proving the fly to be an excellent model for studying ocular human diseases. The OPA1 gene is phylogenetically highly conserved and the organization of the homologous gene in *Drosophila* is less complex than in humans. In the fly, it spans 4,741 bp of genomic sequence and consists of 14 exons. Moreover, we discovered only two splice variants of the fly OPA1 mRNA. For our studies, we are using knock-out flies that carry a P-element insertion in exon 2 of the drosophila OPA1 gene. First studies of this fly line revealed that while heterozygous flies are seemingly perfectly viable, homozygous animals show growth defects and die at a late larval stage. Ectopic overexpression of OPA1 in the fly eye leads to a small eye phenotype, whereas expression in neuronal tissue prevents development into adult flies. The affected pathways producing these developmental impairments will have to be identified.

Selected Publications

Pesch, UEA, Fries, JE, Bette, S, Kalbacher, H, Wissinger, B, Alexander, C, Kohler, K. (2003). OPA1, the disease gene for autosomal dominant optic atrophy, is specifically expressed in ganglion cells and intrinsic neurons of the retina. IOVS 45, 4217–4225.



Ectopic overexpression of dOPA1 in the fly eye results in a small eye phenotype.

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Neuronal Stem Cells

Gerd Kempermann

Stem cells in the adult brain serve an as yet largely unknown function. However, they contribute to cellular brain plasticity, that is the malleable link between the brain's form and function. It is well known that activity is "good for the brain" and that leading an active life has a positive effect, for example by reducing the risk of developing Alzheimer disease. The cellular basis of such activity-dependent plasticity, however, is still hardly known. Despite many suggestive examples of plasticity, the brain heals poorly and many neurological and psychiatric disorders are chronic and irreversible.

One particular case of cellular plasticity is "adult neurogenesis", the generation of new neurons in the adult brain. Adult neurogenesis originates from resident stem cells in the brain. But although neuronal stem cells can be found throughout the brain, adult neurogenesis is limited to two privileged regions in the olfactory system and the hippocampus. The hippocampus is a brain region involved in learning and memory and as such plays a fundamental role in higher cognitive functions. We are interested in how new neurons contribute to normal hippocampal function and if and how a failure of adult neurogenesis might be involved in the pathogenesis of complex disorders like age-related memory loss and cognitive impairment, temporal lobe epilepsy, major depression, and Alzheimer disease. Beyond these hippocampus-specific aims, we can learn from adult hippocampal neurogenesis how the development of new neurons is possible under the conditions of the adult brain. In general, the brain is non- or even anti-neurogenic. Neurogenesis is the interaction between the stem cells and their local microenvironment, the so-called "niche". Our goal is to define the particular properties of stem cells and the neurogenic niche that contribute to adult neurogenesis. Although only few new neurons are generated in the adult and aging brain, the entire molecular machinery that is necessary to allow neuronal development is maintained into old age.

Neuronal development in the adult brain

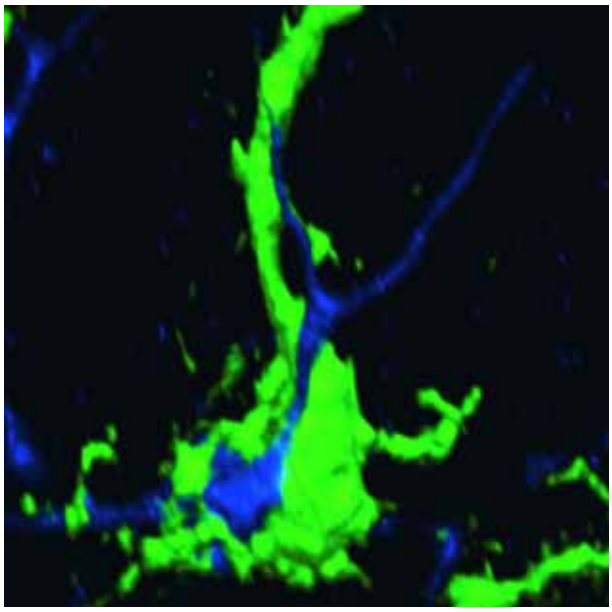
In a series of studies, we characterized neuronal development in the adult hippocampus in detail. The cell of origin is a radial glia-like stem cell. Highly proliferative intermediate progenitor cells allow a rapid expansion of the precursor cell pool in response to neurogenic stimuli. The earliest signs of neuronal determination can be found on the level of these progenitor cells. As in embryonic development, the first synaptic input to newborn neurons is GABAergic which is later exchanged for a predominantly glutamatergic innervation (collaboration with H. Kettenmann). Once the cells have exited the cell cycle, they go through a transient phase with a particular marker profile during which they extend their dendrites and axons. Within this brief period, and thus at a relatively immature stage, the decision is made whether the new neurons are recruited for long-term survival. Actual neuronal maturation follows and takes several weeks. During the immature stages of development, the new neurons show a close spatial association with the radial glia-like stem cells and presumably the other elements of the stem cell niche. We have defined six milestones of neuronal development in the adult hippocampus and identified restriction points at which regulation occurs. The two major aspects of regulation relate to the expansion of the precursor cell pool and of the activity-dependent recruitment of the newborn neurons.

Activity-dependent plasticity in the adult and aging brain

Although the number of stem and progenitor cells decreases with increasing age, adult neurogenesis is maintained into old age. We are using two paradigms of physiological stimulation of neurogenesis which affect two different aspects of neuronal development. Both voluntary wheel running and environmental enrichment increase adult hippocampal neurogenesis. But whereas physical exercise as the presumably less specific stimulus induces precursor cell proliferation, environmental enrichment as the more cognitive and more hippocampus-specific stimulus fosters the survival and recruitment of the new neurons. Both stimuli are effective in old age. As one of the factors that might be responsible for the decrease in neurogenesis in old age, we identified an increase in the expression of corticosteroid receptors on progenitor cells. Our focus lies on the identification of mechanisms by which activity counteracts such age-related changes and thereby preserves the potential for cellular plasticity into old age. Outside the neurogenic region of the adult hippocampus, we found that the reduced response of endogenous brain precursor cells to a brain tumor in the aging brain was associated with reduced survival whereas the implantation of progenitor cells from younger animals improved survival (collaboration with H. Kettenmann).

The molecular bases of adult hippocampal neurogenesis

Natural variation in adult hippocampal neurogenesis is much larger than the effects of regulation found in monogenic models such as transgenic or knockout animals. We are using



The putative stem cells of the adult hippocampus have a surprising, tree-like morphology (green). This appearance resembles radial glia, which play a fundamental role during embryonic brain development. The image shows how a radial glia-like stem cell "cradles" a newborn neuron (blue). The new neuron has already extended processes toward the molecular layer of the dentate gyrus (top) but these have still an immature appearance. The neuron is about 1 week old; the full maturation will take approximately 7 weeks.

recombinant inbred strains of mice to investigate the genetic bases of adult hippocampal neurogenesis. Using "expression genetics", we correlate parameters describing adult hippocampal neurogenesis to data on the transcriptome level. Besides identifying candidate regions (on chromosomes 1 and 5) that might contain quantitative trait genes centrally involved in the control of adult hippocampal neurogenesis, we focus on the analysis of phenotypic and genotypic covariance as a means of elucidating the complex regulatory networks underlying a polygenic quantitative trait like adult neurogenesis.

The research group "Neuronal stem cells" works in close interaction with the independent group "Neurogenic permissiveness", also headed by Gerd Kempermann, funded by VolkswagenStiftung and associated with the Department of Experimental Neurology, Charité University Medicine Berlin.

Selected Publications

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Molecular Neurobiology

Inés Ibañez-Tallon

Ion channels control cellular communication between nerve cells which, in turn, influences neuronal excitability and synaptic transmission. Our group is interested in understanding how ion channel activity determines neuronal function in the mammalian nervous system. We use a combination of molecular, electrophysiology, 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.

Lynx1, an endogenous toxin-like molecule

Lynx1 was discovered in the lab of Prof. Heintz at the Rockefeller University in a screen for novel brain-specific genes. In further studies, we demonstrated that lynx1 encodes a short protein with the cysteine motif characteristic of Ly6 molecules of the immune system and snake α-neurotoxins. This cysteine scaffold determines a particular spatial conformation known as the three-finger toxin fold. lynx1 is highly and specifically expressed in different brain areas. Within these brain structures, lynx1 protein is detected in large projection neurons, where it co-localizes with nicotinic acetylcholine receptors (nAChRs) at the somatodendritic membrane. Biochemical and functional studies further indicated that lynx1 directly associates with nicotinic receptors and modulates their desensitization and electrical conductance. lynx1 discovery is especially intriguing, because it confirms that mammals harbor a natural, endogenous protein resembling snake venom toxins that affects the nervous system.

Tethered toxins

Prompted by the similarities between lynx1 and venom peptide toxins, we have generated recombinant tethered toxins that can be used genetically to target specific ion channels at the cell membrane *in vivo*. These were prepared by cloning synthetic sequences coding for bungarotoxins or conotoxins followed by a short or long linker and the lynx1 hydrophobic sequence for anchoring them to the cell membrane. Electrophysiological analyses proved specific and irreversible block of nicotinic receptors and voltage gated channels, thus providing a new strategy for inactivating ion channels in a cell autonomous manner. *In vivo* studies in zebrafish showed that targeted elimination of nAChR function could be achieved with tethered toxins. We are now using this strategy combined with mouse BAC transgenesis to elucidate the contribution of individual ion channel subtypes underlying the regulation of specific neuronal circuits in the mouse nervous system.

Dynein and mouse development

Our group is interested in the role of cilia in development and disease. We have generated a mouse model with a loss-of-function mutation in the cilia-related molecular motor gene Mdnah5 (also named Dnahc5) that encodes axonemal dynein heavy chain 5 gene. These mice present most of the features of Primary Ciliary Dyskinesia or Kartagener's syndrome, including recurrent respiratory infections, hydrocephalus, and randomization of body situs. Consistently, Mdnah5-deficient mice show tissue-specific ultrastructural and motility defects in cilia of the epithelial cells lining the respiratory tract and in brain ependymal cells. In addition, Mdnah5 is expressed in the ventral surface of the embryonal node where nodal cilia are located and mutant mice exhibit situs inversus in half of the cases. It has been shown that within the node, motile cilia generate the nodal flow that initiates the laterality cascade signaling. We are investigating the role of this dynein in nodal cilia and left-right patterning determination.

Selected Publications

Ibañez-Tallon, I, Wen, H, Miwa, JM, Xing, J, Tekinay, AB, Ono, F, Brehm, P, Heintz, N (2004) Tethering naturally occurring peptide toxins for cell-autonomous modulation of ion channels and receptors in vivo. Neuron, 43(3):305-11.

Ibañez-Tallon, I, Pagenstecher, A, Fliegauf, M, Olbrich, H, Kispert, A, Ketelsen, UP, North, A, Heintz, N, Omran, H. (2004) Dysfunction of axonemal dynein heavy chain Mdnah5 inhibits ependymal flow and reveals a novel mechanism for hydrocephalus formation. Human Molecular Genetics, 13(18):2133-41.

Ibañez-Tallon, I., Heintz, N. and Omran, H. (2003) To beat or not to beat: roles of cilia in development and disease. Human Molecular Genetics, 12: 27-35

Structure of the Group

Group Leader
Dr. Inés Ibañez-Tallon

Scientists
Dr. Silke Frahm
Dr. Beate Liehl

Graduate Students
Martin Laqua, Dipl. Biol.
Annika Stürzbecher, Dipl. Ing

Technical Assistants
Branka Medic, Dipl. Biol.

Secretariat
Sylvia Olbrich

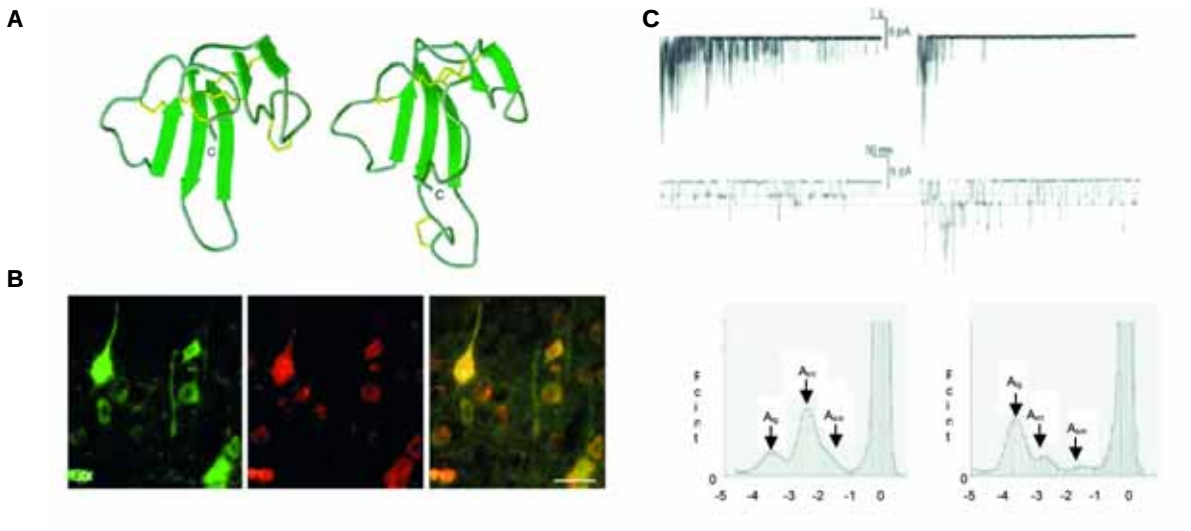


Figure 1. (A) Comparison of the lynx1 model and the snake venom α-bungarotoxin experimental structure. (B) Colocalization of lynx1 and nicotinic receptors (nAChR) in cortical pyramidal neurons. (C) Single channel currents through nAChRs are modulated by lynx1.

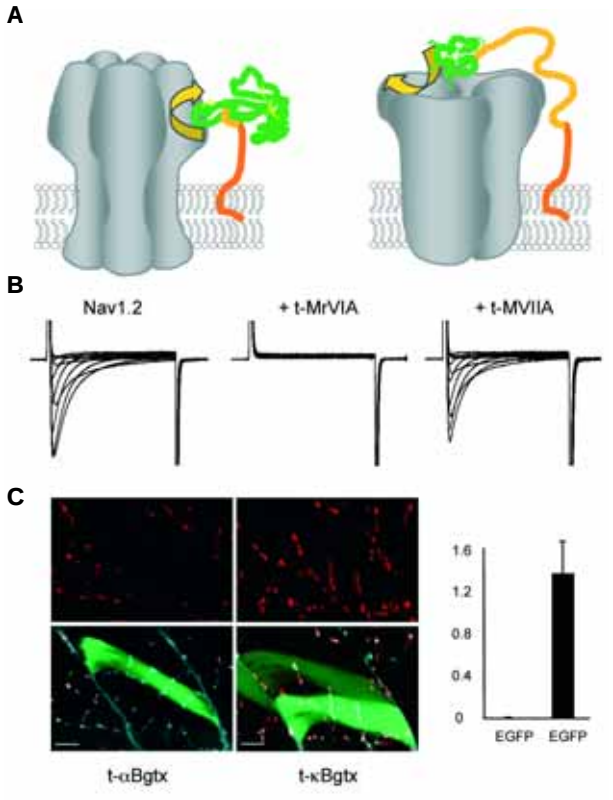


Figure 2. (A) Model representing the mode of binding of tethered toxins to nAChRs (left) or to voltage-gated channels (right), green represents the toxin, yellow the linker and orange the GPI anchor. (B) Representative recordings obtained in oocytes expressing the sodium voltage gated channel Nav1.2 alone or together with tethered conotoxins MrVIA and MVIIA. (C) Functional inactivation of muscle nAChRs in vivo in zebrafish.

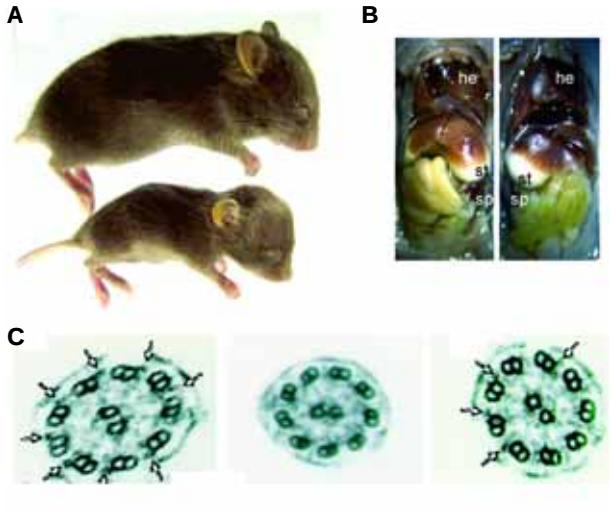
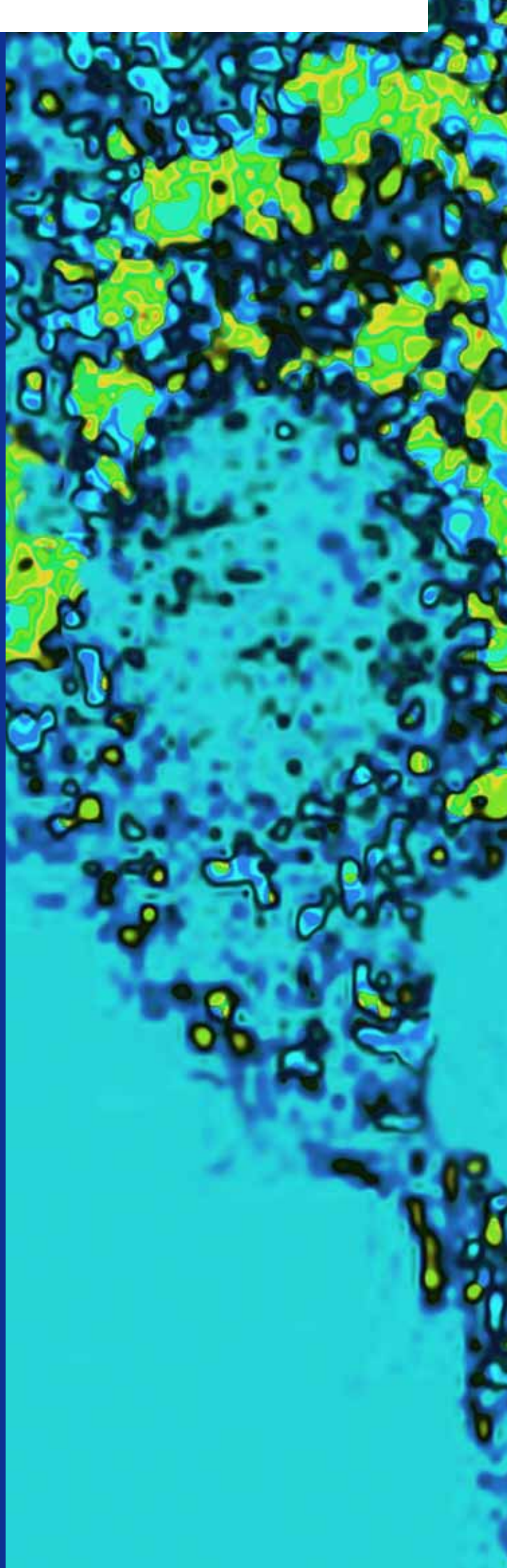


Figure 3. (A) Mdnah5-deficient mice show growth retardation, hydrocephalus, (B) and randomization of body situs. (C) Loss-of-function of Mdnah5 results in total or partial absence of axonemal outer arms.

Academics

Akademische Aktivitäten



Academic Appointments

Berufungen

Appointments at the MDC/Joint Appointments

The MDC has established an official cooperation agreement with the Humboldt University Berlin and the Free University of Berlin 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.

Berufungen an das MDC/Gemeinsame Berufungen

Das MDC hat mit der Humboldt-Universität zu Berlin und der Freien Universität Berlin 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.

Prof. Michael Bader (links) und seine Mitarbeiterin Katja Tenner im Labor im Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch. Photo: Uwe Eising / Copyright: MDC
Prof. Michael Bader (left) and his coworker Katja Tenner in the lab at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch. Photo: Uwe Eising / Copyright: MDC

Max Delbrück, Bronzebüste des Bildhauers Hans Scheib.
Photo: Uwe Eising/Copyright: MDC
Max Delbrück, Bronze bust by Sculptor Hans Scheib.
Photo: Uwe Eising/Copyright: MDC

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 Humboldt University Berlin 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 Free University Berlin in December 2002.

During the period of this report, the following academic appointments were made:

Prof. Peter Daniel, Charité Group Leader und Guest Group Leader at the MDC for Clinical and Molecular Oncology, was appointed in October 2004 by the Charité University Medical Hospital Berlin as a C3/W2 Professor for Clinical and Molecular Oncology.

Prof. Michael Gotthardt, MDC Group Leader "Neuromuscular and Cardiovascular Cell Biology", was appointed in 2004 as a Junior Professor by the MDC and Charité University Medical Hospital Berlin.

Prof. Norbert Hübner, MDC Group Leader "Medical Genomics Research", was appointed in 2005 as C3/W2 Professor

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 Humboldt-Universität 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 Freien Universität Berlin eine Ergänzungsvereinbarung beschlossen.

Im Berichtszeitraum gab es folgende Berufungen:

Prof. Peter Daniel, Charité-Forschungsgruppenleiter und Gastgruppenleiter am MDC für Klinische und Molekulare Onkologie, hat im Oktober 2004 den Ruf der Charité - Universitätsmedizin Berlin auf die C3/W2-Professur für Klinische und Molekulare Onkologie angenommen.

Prof. Michael Gotthardt, MDC-Forschungsgruppenleiter Neuromuskuläre und Kardiovaskuläre Zellbiologie, hat 2004 den Ruf des MDC und der Charité - Universitätsmedizin Berlin auf eine Juniorprofessor angenommen.

Prof. Norbert Hübner, MDC-Forschungsgruppenleiter „Medizinische Genomforschung", hat 2005 den Ruf des MDC und der Charité - Universitätsmedizin Berlin auf eine C3/W2-Professor für Medizinische Genetik und Genomik angenommen. Prof. Hübner war 2004 von MDC und Charité auf eine Juniorprofessur berufen worden.

Dr. Inéz Ibañez-Tallon, Howard Hughes Medical Institute, Rockefeller University, Laboratory of Molecular Biology, New York, NY, USA, hat 2005 den Ruf des MDC als Forschungsgruppenleiterin für Molekulare Neurobiologie angenommen.

Dr. Zsuzsanna Izsvák, MDC-Forschungsgruppe „Transposition" (Dr. Zoltán Ivics), wurde 2004 mit dem zum ersten Mal vergebenen European Young Investigator Award (EURYI) in Stockholm ausgezeichnet. Seit 2005 leitet Frau Izsvák die Forschungsgruppe „Mobile DNA" am MDC.

Prof. Jens Jordan, Charité - Universitätsmedizin Berlin, Campus Buch, Helmholtz-Stipendiat, „Genetik und Pathophysiologie des Herz-Kreislaufsystems" (Prof. Friedrich C. Luft), hat 2004 den Ruf der Charité - Universitätsmedizin Berlin auf eine C3/W2-Professor für Nephrologie und Hypertensiologie angenommen. Gleichzeitig leitet er die klinische Gastgruppe „Erkrankungen des autonomen Nervensystems" am MDC.

Prof. Achim Leutz, MDC-Forschungsgruppenleiter „Zelldifferenzierung und Tumorigenese", hat 2004 den Ruf des MDC und der Humboldt Universität zu Berlin auf die C4/W3-Professur für Molekulare Entwicklungsbiologie und Onkologie angenommen.

for Medical Genetics and Genomics by the MDC and the Charité University Medical Hospital Berlin. Prof. Hübner was appointed as Junior Professor in 2004 by the MDC and the Charité University Medical Hospital Berlin.

Dr. Inéz Ibañez-Tallon, Howard Hughes Medical Institute, Rockefeller University, Laboratory of Molecular Biology, New York, NY, USA, was appointed in 2005 by the MDC as Group Leader for Molecular Neurobiology.

Dr. Zsuzsanna Izsvák, MDC Group Leader "Transposition" (Dr. Zoltán Ivics), was awarded the first European Young Investigator Award (EURYI) in Stockholm in 2004. Since 2005, Dr. Izsvák leads the MDC research group "Mobile DNA".

Prof. Jens Jordan, Charité University Medical Hospital Berlin, Campus Buch, Helmholtz Fellow "Genetics and Pathophysiology of the Cardiovascular System" (Prof. Friedrich C. Luft), was appointed in 2004 as C3/W2 Professor for Nephrology and Hypertensionology by the Charité University Medical Hospital Berlin. He also continues to lead the MDC Clinical Guest Group "Disease of the Autonomous Nervous System".

Prof. Achim Leutz, MDC Group Leader "Cell Differentiation and Tumorogenesis", was appointed in 2004 as C4/W3 Professor for Molecular Developmental Biology and Oncology by the MDC and the Humboldt University Berlin.

Prof. Ferdinand le Noble, Cardiovascular Research Institute Maastricht, Maastricht, Niederlande, accepted in January 2006 an appointment as C3/W2 Professor for by the MDC and the Charité University Medical Hospital Berlin.

Prof. Jens Reich, MDC Group Leader "Bioinformatics", was elected Vice-Chair of the German National Ethics Council on June 23, 2005. He has served as a member of the Council since its founding in 2001.

Dr. Frank Rosenbauer, Harvard Institutes of Medicine, Hematology/Oncology, Boston, MA, USA, was appointed in 2005 as Junior Group Leader for "Cancer Stem Cells and Transcription Factors".

Prof. Claus Scheidereit, MDC Group Leader „Signal Transduction in Tumor Cells", was named in 2004 Professor by the Free University in Berlin.

Prof. Clemens Schmitt, Charité University Medical Hospital Berlin, has directed the Clinical Guest Research Group "Tumor Genetics and Cellular Stress Signals" at the MDC since 2004. In 2004, he was appointed as C3/W2 Professor for Oncology and Hematology by the Charité University Medical Hospital Berlin.

Dr. Ulrike Ziebold, Helmholtz Fellow "Genetics of Tumor Suppression and Metastasis" Research Group „Epithelial Signal Transduction, Invasion, and Metastasis" (Prof. Dr. Walter Birchmeier), was awarded the Marie Curie-Excellence Stipend to establish her own research group at the MDC.

Prof. Ferdinand le Noble, Cardiovascular Research Institute Maastricht, Maastricht, Niederlande, hat zum Januar 2006 den Ruf des MDC und der Charité - Universitätsmedizin Berlin auf eine C3/W2-Professur für Vaskuläre Biologie angenommen.

Prof. Jens Reich, MDC-Forschungsgruppenleiter „Bioinformatik", wurde am 23. Juni 2005 in Berlin zum stellvertretenden Vorsitzenden des Nationalen Ethikrats gewählt. Er ist seit Gründung des Gremiums durch das Bundeskabinett im Jahr 2001 Mitglied im Ethikrat.

Dr. Frank Rosenbauer, Harvard Institutes of Medicine, Hematology/Oncology, Boston, MA, USA, hat 2005 den Ruf des MDC als Nachwuchsgruppenleiter für „Cancer stem cells and transcription factors" angenommen.

Prof. Claus Scheidereit, MDC-Forschungsgruppenleiter „Signaltransduktion in Tumorzellen", wurde 2004 von der Freien Universität Berlin zum außerplanmäßigen Professor ernannt.

Prof. Clemens Schmitt, Charité - Universitätsmedizin Berlin, leitet seit 2004 die klinische Gastsgruppe „Tumorgenetik und zelluläre Stressantworten" am MDC. 2004 hatte er den Ruf der Charité - Universitätsmedizin Berlin auf eine C3/W2-Professur für Onkologie und Hämatologie angenommen.

Dr. Ulrike Ziebold, Helmholtz-Stipendiatin „Genetik der Tumorsuppression und Metastasierung" Forschungsgruppe „Signaltransduktion, Invasivität und Metastasierung von Epithelzellen" (Prof. Dr. Walter Birchmeier) erhielt 2004 ein Marie-Curie-Exzellenz-Stipendium, um ihre eigene Forschungsgruppe am MDC aufzubauen.

Das Walter Friedrich-Haus – eines von insgesamt 3 großen Laborgebäuden des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch.
Photo: Uwe Eising / Copyright: MDC
The Walter Friedrich Building - one of 3 main laboratory buildings at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch.
Photo: Uwe Eising/Copyright: MDC

In addition, the following scientists accepted academic positions at other institutions:

Dr. Susanne Arnold, MDC scientist, was appointed in September 2005 as Group Leader of the Emmy Noether Research Group "Brain Energy Metabolism" at the Medical Faculty of the Rheinisch-Westfälischen Technischen Hochschule (RWTH) in Aachen.

Dr. Ralf Bargou, MDC Group Leader "Hematology, Oncology, and Tumor Immulogy" (Prof. Dr. Bernd Dörken), was appointed by the University Clinic in Würzburg as a C3/W2 Professor for Hematology and Internal Oncology.

Dr. Matthias Friedrich, Director of Cardiac Magnetic Resonance Tomography (MRT) at the Franz Volhard Clinic (Charité University Medical Hospital Berlin/HELIOS Clinics GmbH, Berlin-Buch), was appointed in July 2004 as Professor for Cardiology and Radiology at the University of Calgary, Alberta (Canada).

Prof. Rüdiger von Harsdorf, accepted a double appointment in 2005 at the University of Toronto (Canada): 1) Robert McEwen-Chair in Cardiac Regenerative Medicine, Toronto General Research Institute, and 2) Associate Professor, Department of Medicine, Division of Cardiology, Toronto General Hospital, University Health Network.

Dr. Hans-Christian Hennies, MDC Group Leader "Gene Mapping and Identification in Monogenic and Multifactorial Diseases" (Dr. Peter Nürnberg), was appointed in 2004 as Group Leader at the University of Cologne, Center for Functional Genomics Research (ZFG), Department of Dermatogenetics.

Dr. Matthias Köhler, Helmholtz Fellow „Transport Specificity of alpha-Importins", MDC Research Group "Intracellular Proteolysis" (Dr. Thomas Sommer) and "Genetics and Pathophysiology of the Cardiovascular System" (Prof. Friedrich C. Luft), was appointed in October 2005 as Head of the Center for Kidney and Hypertensive Disorders of the Rehabilitations Clinic Damp.

Dr. Daniel Krappmann, Helmholtz Fellow "Antigen receptor-steered Signal Activation in Lymphocytes", MDC Research Group "Signal Transduction in Tumor Cells" (Prof. Claus Scheidereit), was appointed in 2005 as Head of the Research Group "Signalling Processes in the Immune System" at the Research Center for the Environment and Health (Forschungszentrum für Umwelt und Gesundheit, GSF) in Munich.

Dr. Peter Nürnberg, MDC Group Leader, was appointed in 2004 as a C4/W3 Professor for Genomics at the University of Cologne.

Außerdem nahmen die folgenden Wissenschaftler einen Ruf an andere Institute an:

Dr. Susanne Arnold, MDC-Wissenschaftlerin, arbeitet seit September 2005 als Leiterin der Emmy Noether Forschungsgruppe „Energiemetabolismus in Astrozyten" an der Medizinischen Fakultät der Rheinisch-Westfälischen Technischen Hochschule (RWTH) Aachen.

Dr. Ralf Bargou, MDC-Forschungsgruppe „Hämatologie, Onkologie und Tumorimmunologie" (Prof. Dr. Bernd Dörken), hat den Ruf des Universitätsklinikums Würzburg auf die C3/W2-Professur für Hämatologie und Internistische Onkologie angenommen.

Dr. Matthias Friedrich, Leiter der Kardialen Magnetresonanztomographie (MRT) in der Franz-Volhard-Klinik (Charité - Universitätsmedizin Berlin/HELIOS Kliniken GmbH, Berlin-Buch), hat im Juli 2004 den Ruf der Universität von Calgary, Alberta (Kanada) auf eine Professur für Kardiologie und Radiologie angenommen.

Prof. Rüdiger von Harsdorf hat 2005 den Ruf der Universität von Toronto (Kanada) angenommen. Er erhielt eine Doppelberufung: 1. Robert McEwen-Chair in Cardiac Regenerative Medicine, Toronto General Research Institute und 2. Associate Professor, Department of Medicine, Division of Cardiology, Toronto General Hospital, University Health Network.

Dr. Hans-Christian Hennies, MDC-Forschungsgruppe „Gene Mapping and Identification in Monogenic and Multifactorial Diseases" (Dr. Peter Nürnberg), hat 2004 einen Ruf der Universität zu Köln, Zentrum für Funktionelle Genomforschung, Abt. Dermatogenetik (ZFG) als Gruppenleiter angenommen.

Dr. Matthias Köhler, Helmholtz-Stipendiat „Transportspezifität der alpha-Importine", MDC-Forschungsgruppe „Intrazelluläre Proteolyse" (Dr. Thomas Sommer) und „Genetik und Pathophysiologie des Herz-Kreislaufsystems" (Prof. Friedrich C. Luft), hat im Oktober 2005 die Leitung des Zentrums für Nieren- und Bluthochdruckerkrankungen der Rehaklinik Damp übernommen.

Dr. Daniel Krappmann, Helmholtz-Stipendiat „Antigen-Rezeptor gesteuerte Signalaktivierung in Lymphozyten", MDC-Forschungsgruppe „Signaltransduktion in Tumorzellen" (Prof. Claus Scheidereit), hat 2005 den Ruf der GSF – Forschungszentrum für Umwelt und Gesundheit GmbH, München, als Leiter der Forschungsgruppe „Signalprozesse im Immunsystem" angenommen.

Dr. Peter Nürnberg, MDC-Forschungsgruppenleiter, hat 2004 den Ruf der Universität Köln auf eine C4/W3-Professur für Genomik angenommen.

Awards / Preise 2004–2005

2004

Olav Andersen
Biacore Science Award

Andreas Birkenfeld
Hans J. Dengler-Preis, Paul-Martini-Stiftung

M. Cristina Cardoso
Binder Innovationspreis, Deutsche Gesellschaft für Zellbiologie, Firma Binder

Peter Daniel
Preis der Arbeitsgemeinschaft Internistische Onkologie (AIO), Pfizer PharmaciaOncology

Victor Dzau
Max-Delbrück-Medaille

Friedrich C. Luft
Arthur C. Guyton-Preis, University of Mississippi, Jackson, USA

Zsuzsanna Izsvák
European Young Investigator Award (EURYI), European Heads of Research Councils (EUROHORCS), European Science Foundation (ESF)

Hans Schreiber
Humboldt-Forschungspreis, Alexander von Humboldt-Stiftung

2005

Bernd Dörken und Claus Scheidereit
Deutscher Krebspreis, Deutsche Krebsgesellschaft e. V.

Rainer Glaß und Ralf Synowitz
Young Investigator Award, American Brain Tumor Association

Friedrich Luft
Richard Bright Award, American Society of Hypertension

Sergej Nedospasov
Helmholtz-Humboldt-Forschungspreis, Alexander von Humboldt-Stiftung

Joao Pesquero
Friedrich Wilhelm Bessel-Forschungspreis, Alexander von Humboldt-Stiftung

Bruce Ransom
Humboldt-Forschungspreis, Alexander von Humboldt-Stiftung

Tom Rapoport
Max-Delbrück-Medaille

Peter M. Schlag
Anita- und Cuno-Wieland-Preis

Ulrike Ziebold
Monika-Kutzner-Preis, Berlin-Brandenburgische Akademie der Wissenschaften (BBAW)

Hochdotierter europäischer Förderpreis für die ungarische Nachwuchswissenschaftlerin Dr. Zsuzsanna Izsvák vom Max- Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch. Photo: Uwe Eising/Copyright: MDC
Dr. Zsuzsanna Izsvak, a junior group leader from Hungary at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch, was awarded the prestigious European Young Investigators Award. Photo: Uwe Eising/Copyright: MDC

Dr. Ulrike Ziebold vom Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch. Trägerin des Monika Kutzner-Preises der Berlin- Brandenburgischen Akademie der Wissenschaften. Photo: privat
Dr. Ulrike Ziebold of the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch received the Monika Kutzner Prize of the Berlin-Brandenburg Academy of Sciences. Photo: private.

Helmholtz Fellows

Helmholtz Fellowships at the MDC are intended to allow promising young scientists to carry out their own independent research. Helmholtz 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 12 Helmholtz Fellows.

Helmholtz-Stipendium

Helmholtz-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 Helmholtz-Stipendiaten sollen an bestehende Arbeitsgruppen des MDC Berlin-Buch angegliedert werden. In diesem Rahmen werden ihnen Raum und Infrastruktur sowie ein Sachmittelbudget zur Verfügung gestellt. Die gastgebende Forschungsgruppe garantiert Stipendiaten/Stipendiatinnen 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 12 Stipendiaten gefördert.

International PhD Program

The international PhD program "Molecular Cell Biology" is a joint activity of the Max Delbrück Center (MDC) for Molecular Medicine and the Humboldt University (HU) Berlin. The program provides training and research opportunities for university graduates who wish to obtain a PhD in the fields of Cell Biology, Molecular Biology, Molecular Genetics, Molecular Cardiovascular Research, Cancer Research, Developmental Biology, and Neurobiology. Training and research within the PhD program is interdisciplinary with strong links between basic research and medicine.

Students write a research proposal in the first year and give annual presentations of their progress in the following years. Students are advised by their Research Group Leader and two advisors of their PhD Committee and obtain their PhD degree after approval through the Humboldt University (Dr. rer. nat.) or through their national university.

Eligible are students who have obtained an academic degree comparable to the Masters degree or to the German Diploma. Admission to the MDC-HU PhD program is competitive and decided upon by the Graduate Committee. Financial support via PhD fellowships is provided by the MDC.

During the reported period, the MDC received around 200 applications and accepted 13 and 14 applicants into the program in 2004 and 2005, respectively.

Internationales PhD-Programm

Das Internationale PhD-Programm „Molekulare Zellbiologie" ist ein Gemeinschaftsprojekt des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch und der Naturwissenschaftlichen Fakultät der Humboldt-Universität (HU), Berlin. Es wendet sich an Hochschulabsolventen, die eine Dissertation auf dem Gebiet der Zellbiologie, Molekularbiologie, Molekulargenetik, der molekularen Herz-Kreislauf- sowie Krebsforschung, Entwicklungsbiologie oder Neurobiologie anfertigen wollen. Ausbildung und Forschung in diesem PhD-Programm sind interdisziplinär, wobei eine intensive Verbindung zwischen Grundlagenforschung und Medizin im Vordergrund steht.

Die Studenten verfassen im ersten Jahr einen Forschungsantrag und in jedem folgenden Jahr eine Darstellung ihrer Fortschritte. Sie werden durch ihren Forschungsgruppenleiter und zwei Berater des PhD-Ausschusses unterstützt. Ihren akademischen Grad erhalten sie von der Humboldt-Universität (Dr. rer. nat.) oder der jeweiligen Universität ihres Heimatlandes.

Teilnahmeberechtigt sind Studenten, die bereits einen akademischen Grad besitzen, der mit dem „Master" oder dem deutschen „Diplom" vergleichbar ist. Die Zulassung zum Internationalen PhD-Programm wird in einem kompetitiven Auswahlverfahren durch den Graduiertenausschuss des MDC ermittelt.

Das MDC erhält rund 200 Bewerbungen; 13 bzw. 14 Bewerber sind 2004 bzw. 2005 in das Programm aufgenommen worden. Sie erhalten alle ein Stipendium vom MDC.

Congresses and Scientific Meetings

Kongresse und Wissenschaftliche Tagungen

2004

23. Januar, Neujahrsempfang des MDC

30. Januar bis 1. Februar, Bürgerkonferenz der Ethikgruppe des MDC

19. bis 21. Februar, 28. Jahrestagung der Berliner Chirurgischen Gesellschaft, Prof. Schlag, RRK

12. bis 14.März, Bürgerkonferenz der Ethikgruppe des MDC

21. bis 23. Mai, OECI-Konferenz, Prof. Schlag, RRK

28. bis 30. Mai, Berliner Philharmoniker in Berlin-Buch, Zukunft@BPhil Projekt 15: Belsazars Fest

10. Juni, Seminar der Firma ‚waters' „Proteomics"

11. und 12. Juni, Lange Nacht der Wissenschaften

16. Juni, Verabschiedung des Chefarztes der Augenklinik, PD Dr. med. Möller gemeinsam mit dem Berlin-Brandenburgischen Augenärztetag – HELIOS Klinikum Berlin-Buch mit wissenschaftlicher Konferenz

19. Juni, Bucher Symposium FVK, Prof. Luft

7. und 8. Juli, Festsymposium des FMP zur Einweihung des 900 MHerz Spektrometers

15. Oktober, Cardiovascular and Neuronal Basis of Stroke, Abschiedssymposium Prof. Ganten

20. Oktober, Axon 2, 1. Bucher Kliniksymposium

12. und 13. November, 3. Bucher Hämatologie-Forum, RRK, Prof. Ludwig

24. November, Klinische Demonstration der Bucher pädiatrischen Einrichtungen, HELIOS Klinikum Berlin-Buch

2. und 3. Dezember, Brain Tumors 2004, Prof. Kettenmann

Die Bibliothek des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch mit einer Installation blauer Büsten der Akademie der Künste (1996).
Photo: Uwe Eising/Copyright: MDC
The library of the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch with an art installation of blue busts from the Academy of Arts (1996).
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2005

21. Januar, Neujahrsempfang des Campus

18./19. Februar, DEGUM Kongress, Kinderchirurgische Klinik des HELIOS Klinikum Berlin-Buch

27. Februar, Zwillingstreffen

28. Februar, Meeting „Kompetenz in Medizin“ zum Geburtstag von Dr. Vogel – Neurologie

17. März, Bürgerforum der BBB Management GmbH Campus Berlin-Buch

22./23. April, Laborbaukonferenz, Ralf Streckwall, MDC

25. April, Symposium Stroke/Helix, MDC-Vorstand

27. Mai, Tierhauseinweihung

3. Juni, Abschlussveranstaltung des 2. MDC-Mentoringprogramms

2. Juni, Lecture „Visions for Drug Research“ zum Biotech Kongress

15. Juni, Der perfekte Mensch? Projekttage für Schüler und Schülerinnen der gymnasialen Oberstufe (Träger: Evangelische Akademie, Gläsernes Labor, MDC)

25. Juni, Jazz-Konzert der Philharmonie

22. – 24. September, Curac 2005, Jahrestagung der Deutschen Gesellschaft für Computer- und Roboterassisitierte Chirurgie

21./22. Oktober, Konferenz der Rosa-Luxemburg-Stiftung „Berlin Buch, Die Gesundheitsregion: Tradition – Vision – Gefährdungen“

27. – 29. Oktober, 4th International Symposium on Obesity and Hypertension

Neujahrsempfang 2005 des Campus Berlin-Buch (v. l. n. r.) Prof. Walter Rosenthal, Prof. Walter Birchmeier, Prof. Wieland Huttner, Dr. Thomas Sommer, Prof. Helmut Kettenmann, Staatssekretär Dr. Hans-Gerhard Husung und Prof. Günter Stock. Photo: Thomas Oberländer/Copyright: Helios Klinikum Berlin-Buch
New Year's Reception 2005 on the Campus Berlin-Buch (from left to right): Prof. Walter Rosenthal, Prof. Walter Birchmeier, Prof. Wieland Huttner, Dr. Thomas Sommer, Prof. Helmut Kettenmann, State Secretary Dr. Hans-Gerhard Husung, and Prof. Günter Stock. Photo: Thomas Oberländer/Copyright: Helios Klinikum Berlin-Buch

Seminars

Seminare

2004

Speaker		Institute	Titel of Seminar
Christian	Alzheimer	University of Kiel	Inwardly rectifying K channels, muscarinic M2 receptors and signal processing in the hippocampus
Hinrich	Apken	University of Cologne	Manipulating the T cells immune response: Recombinant immunoreceptors endow T cells with predefined specificity
Ernest	Arenas	Karolinska Institute Stockholm, Sweden	Induction of dopaminergic neurons from neural precursors and stem cells
Helga	Bernhard	TU Munich	Isolation and expansion of antigen-specific cytotoxic T cell clones for adoptive immunotherapy
Daniel	Besser	The Rockefeller University, New York, USA	TGF-beta signalling in human embryonic stem cells
Tobias	Bonhoeffer	Max Planck Institute of Neurobiology, Martinsried	Synaptic plasticity and its morphological correlates
Nancy	Bonini	University of Pennsylvania, USA	Drosophila as a model for human neurodegenerative disease:
Allan	Bradley	The Wellcome Trust Sanger Institute, Cambridge, England	Tumor Suppressor Knockouts: A gene and a genome based analysis
Cord	Brakebusch	Max Planck Institute for Biochemistry, Martinsried	Function of Integrins and Rho GTPases in vivo: Adhesion, Migration and more
Thomas	Brand	TU Braunschweig	Functional Characterization of the Popeye Gene Family

Speaker		Institute	Titel of Seminar
Stefan	Brocke	Hebrew University, Jerusalem, Israel	Active recruitment of lesion-inducing lymphocytes to the CNS by constitutive chemokines
Steve	Brown	Genome Centre Oxford, England	New Mouse Models for Human Genetic Disease
Paolo	Cesare	Universita' degli Studi di Roma "La Sapienza", Rome, Italy	PKC-dependent sensitization of mechanical evoked currents in sensory neurons: an in vitro model for the study of pain
Bruce	Edgar	Fred Hutchinson Cancer Research Center Seattle, USA	Growth and Cell Cycle Control in Drosophila
Lea	Eisenbach	Weizmann Institute of Science Israel, Rehovot, Israel	Tumor Associated Antigen peptides and anti-tumor vaccines
Martin	Falcke	Hahn-Meitner Institute, Berlin	Reading Calcium Wave Patterns
Tamas	Freund	Hungarian Academy of Sciences Budapest, Hungary	Mechanisms of cannabinoid actions in the cerebral cortex: implications for anxiety
Thomas	Gasser	University of Tübingen	Genetics of movement disorders: implications for pathogenesis
Walter J.	Gehring	Biozentrum, University of Basel, Switzerland	A deeper Look into Development and Evolution of Eyes and Photoreceptors
Darren	Gilmour	Max Planck Institute, Tübingen	Hierarchical guidance cues coordinate cell movements in the zebrafish sensory nervous system
Robert D.	Goldman	Northwestern University, Chicago, USA	Nuclear lamins: dynamic elements of nuclear architecture
Christo	Goridis	Ecole Normale Superieure Paris, France	From the control of neuro-genesis to autonomic disorders: the role of the Phox2 transcription factors
Günter	Hämmerling	Deutsches Krebsforschungszentrum (DKFZ), Heidelberg	Control of tumor immunity by the microenvironment
Jan	Hoeijmakers	Erasmus University, Rotterdam, Netherlands	A dynamic view on DNA damage repair, transcription and replication in mammalian cells
Roland	Jahns	Medical Polyclinic of the University Würzburg	The role of autoantibodies against the beta1-receptor in cardiomyopathie
Andreas	Jenny	Mount Sinai School of Medicine, New York, USA	Regulation of Fz-planar polarity signaling in Drosophila
E. Yvonne	Jones	Universität Oxford, England	Pegs, pits & portals: A structural biologists's view of TCR recognition
Benno	Jungblut	University of California, San Francisco, USA	A Cellular Framework for Zebrafish Heart Morphogenesis

Speaker		Institute	Titel of Seminar
Lukas	Kenner	University of Graz, Austria	The role of JunB in blood and bone development
Andreas	Kispert	University of Hannover	Vertebrate Segmentation: From Somites to Vertebrae
Ansgar	Klebes	University of California, San Francisco, USA	A genomic analysis of Drosophila imaginal disc development using micro-arrays
Mark	Knuepfer	St. Louis University, School of Medicine, St. Louis, USA	CNS Mechanisms Responsible for Stress- or Cocaine-induced Cardiovascular Disease
Michael	Kühl	University of Ulm	Wnt Signaling during Embryonic Development
Jürgen	Laufs	Ingenium Pharmaceuticals AG, Munich	INGENOtyping – fast generation of allelic variants for gene functional analysis and target validation in mice and rats
Victor	Levenson	Northwestern University, Chicago, USA	Multiplexed analysis of promoter methylation for cancer diagnosis
Olle	Lindvall	University Hospital Lund, Sweden	Stem cell therapies for neurodegenerative disorders--will they ever work
Hartmut	Luecke	University of California, Irvine, USA	Light-driven ion-pumping and signaling in microbial rhodopsins
Hiroshi	Maruta	Ludwig Institute for Cancer Research, Melbourne, Australia	Signal therapy of cancers by blocking the PAK1 pathway
Gerd	Multhaup	Free University Berlin	The metallobiology of the amyloid precursor protein of Alzheimer's disease
Sergei	Nedospasov	Engelhardt Institute of Molecular Biology, Moscow, Russia, and National Cancer Institute, Frederick, USA	Role of tumor necrosis factor and lymphotoxin in host defense and cancer
Hitoshi	Okazawa	Tokyo Metropolitan Institute of Neuroscience, Tokyo, Japan	Toward understanding of nuclear responses to polyglutamine disease proteins
Slavko	Pecar	Jozef Stefan Institute, Ljubljana, Slovenia	Biologically active nitroxides
Rainer	Pepperkok	European Molecular Biology Laboratory, EMBL, Heidelberg	Imaging the dynamics and interaction of vesicular coat complexes in living cells
Stefan	Pulst	David Geffen School of Medicine, University of California Los Angeles (UCLA), USA	Spinocerebellar ataxia type 2: Of mice (worms) and men
Tom	Rapoport	Harvard University, Boston, USA	Structure and function of a protein-conducting channel
Charles	Ribak	University of California, Irvine, USA	Newborn neurons in the epileptic brain: Abnormal dendrites and altered migration

Speaker		Institute	Titel of Seminar
Klaus	Rohr	University of Cologne	The role of the vascular system in thyroid induction and morphogenesis
Wolfgang	Rottbauer	University of Heidelberg	Dissection of genetic components of cardiovascular function in zebrafish
Wolf	Singer	Max Planck Institute for Brain Research, Frankfurt	The role of neuronal synchrony in signal processing and learning
Markus	Stoffel	The Rockefeller University, New York, USA	The role of forkhead transcription factor HNF-3beta/Foxa2 in metabolism and diabetes
Jens	Tampe	Ascenion GmbH, Munich	Intellectual Property on Research Tools
Jacqueline	Trotter	University of Mainz	Wrapping axons: Molecular events in glial-axon interaction and myelination
Lyubomir	Vassilev	Hoffmann-La Roche Inc., Nutley, New Jersey, USA	Pharmacological intervention in p53 regulation by small-molecule antagonists of MDM2
Andrea	Vortkamp	Max Planck Institute for Molecular Genetics, Berlin	Molecular Control of Chondrocyte Differentiation
Hartmut	Wekerle	Max Planck Institute of Neurobiology, Martinsried	Brain Watching – Imaging and functional characterization of autoimmmue T lymphocytes in the central nervous system
Sabine	Werner	Institute of Cell Biology, Eidgenössische Technische Hochschule (ETH), Zurich, Switzerland	Fibroblast growth factors and activin: important regulators of inflammation and tissue repair
Robert W.	Williams	University of Tennessee, Memphis, USA	The Complex Genetics of Brain Structural and Functional Variation
Jochen	Wittbrodt	European Molecular Biology Laboratory, EMBL, Heidelberg	Control of proliferation and differentiation in the developing retina
Wolfgang	Wurst	GSF - National Research Center for Environment and Health, Neuherberg	Genetic control of mesencephalic dopaminergic neuron development
Darryl	Zeldin	NIH, National Institute of Environmental Health Sciences, NIEHS, North Carolina, USA	Cytochrome P450 Epoxygenase Metabolites and Cardiac Function
Andreas	Zimmer	University of Bonn	Neuromodulators and stress-induced behaviours: New insights from knockout mice
Frauke	Zipp	Charité - Universitätsmedizin, Berlin	Immune cell mediated damage mechanisms in brain autoimmunity

2005

Speaker		Institute	Titel of Seminar
Kirill	Alexandrov	Max Planck Institute of Molecular Physiology, Dortmund	Can trypanosomatid parasites help us to unclog the pipelines of structural genomics projects?
Thomas	Biederer	Yale University, New Haven, USA	Synaptogenesis in the brain: The roles of the adhesion molecules SynCAM and Neuroligin
Anne	Boullerne	University of Illinois, Chicago, USA	Lineage of Adult Human Oligoden-drocytes
James	Brisco	National Institute for Medical Research, NIMR, London, England	Signals, Gradients and the Control of Neural Cell Fate
Margaret	Buckingham	Institute Pasteur, Paris, France	A tale of two Pax genes - from skeletal to smooth muscle
József	Burgyán	Agricultural Biotechnology Research Center, Gödöllo, Hungary	Defence and counter defence: The mechanism of RNA silencing and its role in the virus host interaction
Toni	Cathomen	Charité - Campus Benjamin Franklin, Virology Department, Berlin	Custom zinc-finger nucleases to stimulate targeted modifications of the genome
Catherine	Dargemont	Institut Jacques Monod, Paris, France	Synchronization between transcription and mRNA nuclear export: Role of ubiquitin and UBA domains
Ben	Davies	Genoway Germany GmbH, Hamburg	Genetically modified rodents: time saving solutions and new relevancy

Die Bibliothek des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch.
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The library of the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch.
Photo: Uwe Eising/Copyright: MDC

Speaker		Institute	Titel of Seminar
Leon J.	De Windt	Interuniversity Cardiology Institute Utrecht, Netherlands	Calcium-dependendent transcription factors in cardiac hypertrophy and failure
Ole	Doering	University of Bochum	Biomedical Research and Ethical Regulations in China. Some Observations about Gene Therapy, Human Embryo Research, Assisted Reproduction and Struggles of Interest
Shumin	Duan	Chinese Academy of Sciences Shanghai, China	Purine receptor mediated neuron-glia interactions
Michael R.	Duchen	University College London, England	Roles of calcium, mitochondria and oxidative stress in the neurotoxicity of beta amyloid
Martin	Duennwald	The Whitehead Institute for Biomedical Research, Cambridge, MA, USA	Using Yeast to Decipher Polyglutamine Toxicity and Aggregation
Michael	Elbaum	Weizmann Institute of Science, Rehovot, Israel	Gene Delivery by Agrobacterium: Studies of the VirE2-ssDNA Interaction
Felix B.	Engel	Harvard Medical School, Boston, USA	Differentiated Mammalian Cardiomyocytes Can Proliferate: A Platform for Myocardial Repair?
Douglas	Feinstein	University of Illinois, Chicago, USA	Noradrenergic Modulation of Brain Inflammation
Dan	Finley	Harvard Medical School, Boston, USA	Regulators of the proteasome
Thomas	Floss	GSF - National Research Center for Environment and Health, Neuherberg	19,000 pre-fabricated knockouts: functional analysis of the mouse genome by gene trappin
Lyko	Frank	Deutsches Krebsforschungszentrum (DKFZ), Heidelberg	Functional characterization of DNA methylation in Drosophila
Magdalena	Götz	GSF - National Research Center for Environment and Health, Neuherberg	Glial cells generate neurons: neural stem cells and the master regulator Pax6
Mariam	Grigorian	Danish Cancer Society, Copenhagen, Denmark	Functional significance of S100A4 (Mts1) in tumor-stroma interplay
Phillip	Grote	Dartmouth Medical School, Department of Genetics, Hanover, NH, USA	The Analysis of the tra-4/PLZF-like Gene Uncovers the Role of Epigenetic Gene Regulation in C. elegans Sex Determination
Christine	Hartmann	Research Institute for Molecular Pathology, IMP, Vienna, Austria	Osteoblast or chondrocyte – β-catenin levels matter
Volker	Haucke	Institute for Chemistry, Free University Berlin	Regulatory mechanisms in clathrin-mediated endocytosis
Adrian	Hayday	King's College London, England	The regulation of tissue immunosurveillance by NKG2D-ligands

Speaker		Institute	Titel of Seminar
Mirjam	Heemskerk	Leiden University, Netherlands	The functional activity of genetically engineered T cell receptor transferred T cells is highly dependent on pairing properties of the transferred TCR alpha and beta chains
Carl-Philipp	Heisenberg	Max Planck Institute of Molecular Cell Biology and Genetics, Dresden	Adhesion dynamics in germ layer formation during zebrafish gastrulation
Kay	Hofmann	Memorec GmbH, Cologne	A bioinformatical journey to the origins of the ubiquitin system
Dirk	Homann	University of Colorado, USA	Regulation of antiviral CD8+ and CD4+ T cell memory
Thorsten	Hoppe	University of Hamburg	Regulation of the Myosin-Directed Chaperone UNC-45 by a Novel Ubiquitylation Complex
Markus	Hoth	University of Homburg	Calcium dependent activation of T-lymphocytes
Z. Josh	Huang	Cold Spring Harbor, New York, USA	Constructing GABAergic circuits in Neocortex and Cerebellum: from subcellular synapse targeting to activity-dependent plasticity
Berthold	Huppertz	Dept. of Anatomy, University of Aachen	Trophoblasts and vascular remodelling in the placenta
Wieland	Huttner	Max Planck Institute of Molecular Cell Biology and Genetics, Dresden	The Cell Biology of Neurogenesis
Peter	Jonas	University of Freiburg	Presynaptic ion channels in hippocampal mossy fiber synapses
Thomas	Joos	NMI Natural and Medical Sciences Institute at the University of Tübingen	Protein microarrys: technologies and applications
Willi Alfred	Kalender	Universität Erlangen-Nürnberg Institute for Medical Physics	Micro-CT: New Developments and Applications
Ole	Kiehn	Karolinska Institute Stockholm, Sweden	Physiologic and genetic deciphering of locomotor circuits in mammals
Sergei	Kirischuk	Humboldt University Berlin	GABAB- and A1-receptors control GABAergic synaptic transmission in the layer I of the developing mouse visual cortex
Joachim	Kirsch	University of Heidelberg	Determination of inhibitory synaptogenesis in time and space
Janina	Kneipp	Harvard Medical School, Boston, USA	Vibrational spectra from tissues, cells, and subcellular structures
Elisabeth	Knust	University of Düsseldorf	From epithelial cell polarity to retinal degeneration: lessons from Drosophila

Speaker		Institute	Titel of Seminar
Carla	Koehler	University of California, Los Angeles, USA	Biogenesis of the mitochondrial inner membrane
Bernhard	Korn	RZPD Deutsches Ressourcenzentrum für Genomforschung, Berlin	RZPD: Resources for the analysis - of gene silencing, gene regulation and protein expression
Thomas	Kuner	Max Planck Institute for Medical Research, Heidelberg	Spatial organization and dynamics of synaptic vesicles in the calyx of Held
Christoph	Lengauer	Sidney Kimmel Comprehensive Cancer Center, Baltimore, USA	Chromosome Acrobatics - Genetic instability in cance
Ferdinand	le Noble	Cardiovascular Research Institute (CARIM), Maastricht, Netherlands	Convergence of hemodynamics and neural genes in cardiovascular development
Klaus	Ley	University of Virginia Health System, Virginia, USA	Arrest Chemokines and Monocyte Recruitment to Atherosclerotic Lesions; Neutrophil Homeostasis
Jochen C.	Meier	Charité - Universitätsmedizin Berlin	Epilepsy, new therapeutic possibilities emerging from editing of glycine receptor mRNAs
Michael	Meisterernst	Geneexpression Department, GSF - National Research Center for Environment and Health, Neuherberg	Mechanisms of Gene Control in Chromatin
Anming	Meng	Department of Biological Sciences and Biotechnology Tsinghua University, Peking, China	Angiomotin-like 2 is involved in cell migration and embryonic patterning in ze
Ute	Moll	Stony Brook University, New York, USA	The pro-apoptotic mitochondrial p53 program - A link between inflammation, cancer and chromatin remodelling
Dirk	Montag	Leibniz Institute for Neurobiology, Neurogenetics, Magdeburg	Learning, Memory, and Information Processing in Mouse Mutants
Hannah	Monyer	University of Heidelberg	Genetic approaches to study network activity
Diana	Moss	University of Liverpool, England	Ig LONs, Diglons: from growth cones to cancer
Gerd	Multhaup	Free University Berlin	A role for copper in Alzheimer’s disease
Harald	Neumann	University of Bonn	Molecular mechanism and therapy of inflammatory neurodegeneration
Mami	Noda	Kyushu University, Fukuoka, Japan	Physiological and molecular biological characterization of KCNQ channels in neuron and glia
Baldomero M.	Olivera	University of Utah, USA	Conotoxins: from fish-hunting cone snails to ion channels and drug development

Mittagspause auf der Terrasse der Mensa.
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Lunch on the cafeteria patio.
Photo: Uwe Eising / Copyright: MDC

Speaker		Institute	Titel of Seminar
Arno	Pähler	Protein Data Bank at Osaka University, Japan	Visualization of structural information with xPSSS
Sarah	Perrett	Institute of Biophysics Chinese Academy of Sciences, Peking, China	The Yeast Prion Protein Ure2: Function, Folding and Amyloid Formation
Jean Claude	Perriard	Institute for Cell Biology, Zurich, Switzerland	Cytoarchitecture and Cardiomyocyte Functions: Is the M-band good for anything?
Regine	Peschka-Süss	Albert Ludwigs-Universität, Freiburg	Cellular uptake and intracellular trafficking of particulate drug delivery systems
Maja	Petkovic	University of Zurich, Switzerland	The Rothmund-Thomson syndrome gene product, RECQL4, controls genome stability
Tatiana	Petrova	University of Helsinki, Finland	Control of lymphatic vascular development
Frank	Pfrieger	Centre de Neurochimie, Strasbourg, France	Influence of glial cells on neuronal differentiation
Dajun	Qian	City of Hope National Medical Center, Duarte, California, USA	Haplotype Reconstruction in Population Individuals using Coalescent Trees
Bernhard	Radlwimmer	Deutsches Krebsforschungszentrum (DKFZ), Heidelberg	Tumorgenetic screening using CGH to microarrays

Das Torhaus mit dem Café Max.
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The Gatehouse with the Café Max.
Photo: Uwe Eising/Copyright: MDC

Speaker		Institute	Titel of Seminar
Katja	Rateitschak	University of Rostock	Mathematical modeling and simulation of the Wnt signaling pathway
Wilfried	Roth	Deutsches Krebsforschungszentrum (DKFZ), Heidelberg	Death effector domain proteins in malignant brain tumors
Mauro	Santibanez-Koref	Institute of Human Genetics University of Newcastle, England	Genetic components of O6-alkylguanine-DNA alkyltransferase expression variability
Julio	Scharfstein	Federal University of Rio de Janeiro, Brasil	Bradykinin B2 receptors of dendritic cells: Innate immunity sensors of danger in infection by kinin-releasing parasites
Ralf	Schnabel	TU Braunschweig	4D-microscopy & some results: Fate, migrations, form & curious animals
Jost	Schönberger	University of Würzburg	Mutation in the transcriptional coactivator EYA4 causes dilated cardiomyopathy and sensorineural hearing loss
Jörg	Schulz	University of Göttingen	Parkinson's disease: from genes to treatment
Gerald G.	Schumann	Paul-Ehrlich-Institut, Federal Agency for Sera and Vaccines, Langen	Germ Line Expression of the Mobile Human LINE-1 Retrotransposon is Both Shaping the Genome and Causing Disease
Matthias	Selbach	Max Planck Institute for Biochemistry, Munich	Tyrosine phosphorylated bacterial effector proteins: the enemies within

Speaker		Institute	Titel of Seminar
Lilianna	Solnica-Krezel	Vanderbilt University Nashville, USA	Genetic regulation of convergence and extension gastrulation movements in zebrafish
Lukas	Sommer	Eidgenössische Technische Hochschule (ETH), Zurich, Switzerland	Growth factors regulating neural stem cell development
Frank	Sprenger	University of Cologne	Regulation of entry into and exit from mitosis in Drosophila
Jörg	Stehle	University of Frankfurt	Melatonin - mechanisms for coding and decoding a signal for circadian time
Junji	Takeda	Osaka University, Japan	New strategy to analyze gene functions in mice
Manuel	Than	Max Planck Institute for Biochemistry, Martinsried	Structures of the proprotein convertase (PC) family of proteinases: Biological and pharmacological implications
Zoltán	Varga	University of Oregon, Eugene, USA	Notch signalling specifies anterior pituitary cell types in zebrafish
Michael	Veit	Vet.-Med. Faculty, FU Berlin	Protein palmitoylation and vesicular trafficking – Identification of proteins with (self)palmitoylating activity
Eric	Verdin	Gladstone Institute of Virology and Immunology, San Francisco, USA	Reversible protein acetylation: A posttranslational modification with multiple biological functions
Maria	Wartenberg	GKSS, Geesthacht	In vitro models for tumor induced angiogenesis
Anke	Witting	University of Washington, USA	The Role of the Endocannabinoid Signaling System in Neuroinflammation
David P.	Wolfer	University of Zurich, Switzerland	Molecular and cellular cognition: perspectives and limits of mouse models
Gregory	Wulczyn	Charité - Universitätsmedizin Berlin	Activity of two conserved microRNA, let-7 and mir-125, during neural differentiation
Wolfgang	Zachariae	Max Planck Institute of Molecular Cell Biology and Genetics, Dresden	Reproduction by destruction: meiosis-specific regulation of the anaphase-promoting complex
Hanns Ulrich	Zeilhofer	University of Zurich, Switzerland	Molecular determinants of spinal pain control

Overview

Überblick

Overview

Creation of a Translational Research / Experimental and Clinical Research Center (ECRC)

The MDC and Charité have proposed to found and build an Experimental and Clinical Research Center (ECRC) on the Berlin Buch Campus. Berlin Buch provides an ideal environment for translational research since basic science research institutes (Max Delbrück Center for Molecular Medicine, MDC, and Research Institute for Molecular Pharmacology, FMP), university-affiliated clinical departments (Charité and HELIOS Clinics), and biotechnology companies are all situated on the same campus.

Combining basic and clinical research is a tradition in Berlin Buch that dates back for almost 100 years. The MDC has fostered collaborations between basic and clinical scientists since its foundation in 1992. Clinicians conduct research in MDC laboratories, where they have access to state-of-the-art molecular biology. Conversely, MDC researchers are exposed to relevant medical questions, learn about the current possibilities or limitations of diagnostics and treatment, and participate in the clinical research. Such collaborations have already led to new discoveries in translational research. If expanded and optimally supported, these interdisciplinary activities will allow translational research at an accelerated pace. The designated location of the ECRC between the MDC/FMP research centers and the Charité/ HELIOS clinics will facilitate collaborative interactions with the aim to establish new diagnostic and therapeutic procedures. The traditional MDC research focus, cardiovascular disease, cancer, and neurological disease, will be pursued at the ECRC. Cardiovascular research in particular will be expanded.

The ECRC will be built on the site of the present Robert Rössle Cancer Clinic. The ECRC will provide 3100 m² of research space in total which will be reserved for:

- regular laboratories, so-called interface laboratories, and offices for selected translational research projects.
- a combined Optical Microscopy and Macroscopic Optical Imaging Facility.

Überblick

Errichtung eines Zentrums für Translationsforschung / Experimental and Clinical Research Center (ECRC)

Das MDC und die Charité wollen ihre Zusammenarbeit ausbauen und planen, ein Zentrum für Translationale Forschung, das Experimental and Clinical Research Center (ECRC) auf dem Campus Berlin-Buch zu errichten. Das ECRC wird den Austausch wissenschaftlicher Ideen zwischen Labor und Klinik verstärken und auf diese Weise die Übertragung (Translation) wissenschaftlicher Erkenntnisse in die klinische Anwendung beschleunigen. Berlin-Buch bietet ideale Voraussetzungen für translationale Forschung, da sich sowohl Grundlagenforschung (MDC und FMP – Forschungsinstitut für Molekulare Pharmakologie) und klinische Forschung (Charité und HELIOS Kliniken GmbH) als auch eine Vielzahl an Biotechnologiefirmen auf dem Campus befinden.

Die Verbindung von Grundlagenforschung und klinischer Forschung hat in Berlin-Buch eine fast 100-jährige Tradition. Seit der Gründung des MDC im Jahre 1992 werden Kooperationen zwischen Grundlagenwissenschaftlern und Klinikern gefördert. Kliniker arbeiten in den MDC-Forschungslaboren und haben auf diese Weise einen direkten Zugang zu den neuesten Entwicklungen auf dem Gebiet der Molekularbiologie, während MDC-Wissenschaftler mit medizinisch relevanten Fragestellungen konfrontiert werden und so die derzeitigen Möglichkeiten in Diagnostik und Behandlung von Krankheiten kennen lernen. Dieser intensive Wissensaustausch hat bereits zu neuen Entdeckungen geführt. Die Ausweitung und optimale Förderung dieser interdisziplinären Aktivitäten wird die translationale Forschung beschleunigen.

Das ECRC wird auf dem Gelände der bestehenden Robert-Rössle-Krebsklinik errichtet, zwischen den Forschungsinstituten MDC/FMP und den Charité/HELIOS Kliniken, und so eine zusätzliche Schnittstelle zwischen den Bereichen schaffen. Das Ziel dieser Zusammenarbeit ist es, neue Ansatzpunkte in der Diagnostik und Therapie der häufigsten menschlichen Krankheiten zu finden. Die traditionellen For-

Das Max Delbrück Communications Center (MDC.C). Davor das zum Skulpturenpark des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch gehörende „Große Sonnenzeichen I“ von Rainer Kriester.
Photo: Uwe Eising / Copyright: MDC
The Max Delbrück Communications Center (MDC.C). In the foreground, the sculpture “Large Sun Sign I” by Rainer Kriester. Photo: Uwe Eising / Copyright: MDC

- an experimental (animal) part of a Ultrahigh Field MRT Facility.
- a clinical part (human applications) of a Ultrahigh Field MRT Facility.
- general infrastructure.

Investments for the construction and equipment of the ECRC have been applied for via the Helmholtz-Association and the Federal Ministry of Education and Research (BMBF). The ECRC will provide research facilities for approximately 70 investigators and assistants, third-party funded personnel and guest scientists included. Running costs are funded from the institutional funding of the MDC and partially from third-party revenues. The MDC makes 5 million € from its budget position “Zuweisungen und Zuschüsse” available for Translational Research: 1 million € of which will be spent on the clinical training program (KAP) and 1 million € on the clinical cooperation projects (KKP). Three million € will be the research budget of the ECRC. The appointment of a W2 professorship will be funded from the regular budget. In addition, the MDC is campaigning for a professorship position (W3) dedicated to biomedical and physical high field MRI research.

Furthermore, the Charité plans to invest 15 million € from funds of the University Building Promotion Program (HBFG) for the establishment of 3100 m² of research space at the D2 Building in order to replace the existing clinical research

schungsschwerpunkte des MDC, Herz-Kreislauferkrankungen, Krebs und Neurodegenerative Erkrankungen, werden im ECRC weitergeführt, wobei insbesondere die Herz-Kreislaufforschung verstärkt wird.

Das ECRC wird insgesamt 3100 m² neuer Forschungsflächen bereitstellen, die für folgende Anwendungen vorgesehen sind:

- Labor- und Büroflächen für ausgewählte translationale Forschungsprojekte,
- eine Forschungs- und Serviceeinheit für mikroskopische und makroskopische bildgebende Verfahren,
- eine Forschungs- und Serviceeinheit für Ultrahochfeld-Magnet-Resonanz-Bildgebung für tierexperimentelle und klinische experimentelle Applikationen,
- generelle Infrastruktur.

Die Investitionsmittel für den Bau und die Ausstattung des ECRC sind bei der Helmholtz-Gemeinschaft und dem Bundesministerium für Bildung und Forschung (BMBF) beantragt. Im ECRC wird Platz für ca. 70 Wissenschaftler und technisches Personal, sowie drittmittelfinanziertes Personal und Gastwissenschaftler zur Verfügung stehen. Die laufenden Kosten werden durch das MDC und durch Drittmittel getragen. Das MDC stellt seit seinem Bestehen 5 Mill. € seines Budgets als „Zuweisungen und Zuschüsse“ für die klinische Forschung zur Verfügung: 1 Mill. € fließt in das klinische Ausbildungsprogramm (KAP) und 1 Mill. € in die klinischen Kooperationsprojekte (KKP). 3 Mill. € werden das Forschungsbudget des ECRC ausmachen.

Die Charité wird 15 Mill. € aus dem Hochschulbauförderungsprogramm (HBFG) für die Errichtung von weiteren 3100 m² an Forschungsflächen im D2-Teil des Klinikneubaus der HELIOS Kliniken zur Verfügung stellen. Diese Flächen schaffen Ersatz für die existierenden klinischen Forschungslabore in der Franz-Volhard-Klinik und Robert-Rössle-Klinik, die entfallen, wenn die Kliniken in das neue Klinikgebäude umziehen, und Raum für den patientenbezogenen Teil der ECRC-Projekte. Sie werden durch die Etablierung eines klinischen Forschungszentrums nach dem Vorbild amerikanischer „Clinical Research Center“ neu und modellhaft organisiert.

Geleitet wird das ECRC von einem Koordinator. Der Vorstand des MDC wird den Koordinator des ECRC bei seinen Aufgaben unterstützen. Die Durchführung der Kooperationsprojekte, geleitet von MDC- oder Charité-Wissenschaftlern oder neu zu berufenen Wissenschaftlern oder Klinikern mit dem Ziel, Diagnose- und Therapieverfahren zu verbessern und neue zu entwickeln, wird durch das ECRC hervorragend ermöglicht. Die Entscheidung über die Projekte, die im ECRC umgesetzt werden, erfolgt aufgrund einer wissenschaftlichen Begutachtung durch ein aus MDC- und Charité-Wissenschaftlern sowie externen Wissenschaftlern besetztes Komitee. Die Forschung des ECRC wird wie die Forschung am MDC einer regelmäßigen (alle 3 bis 5 Jahre) Evaluation durch externe Gutachter unterliegen.

facilities at the Franz Volhard Clinic and the Robert Rössle Clinic after these clinics have been incorporated in the new HELIOS Clincs. This building will also provide the ideal accommodation for the existing GMP facility, the tumor bank, a general Clinical Research Center, and patient-related clinical parts of the ECRC projects.

A coordinator of translational research, either a clinical or a basic scientist, will head the ECRC. The existing MDC administration and the MDC scientific and administrative directors will support the coordinator in the management of the ECRC. Regarding the daily business of the ECRC, the coordinator will consult with the ECRC group leaders. C4/W3 and C3/W2 professorships will be established at the ECRC that will provide a stable core of personnel. Twinning projects (cooperation between basic and clinical scientists) will be supported at the ECRC that are lead by established MDC or Charité scientists, or by newly appointed scientists or clinicians. The decision to include a twinning project into the ECRC will be done on scientific grounds after evaluating the research. Twinning projects at the ECRC are based on collaborations between basic and clinical scientists and aim to improve diagnosis and therapy. Cooperative projects will be judged on their scientific merit by due process by a standing committee of MDC and Charité faculty and appointed advisors. The ECRC and the MDC provide for regular (3-5 year) external peer review of research activities.

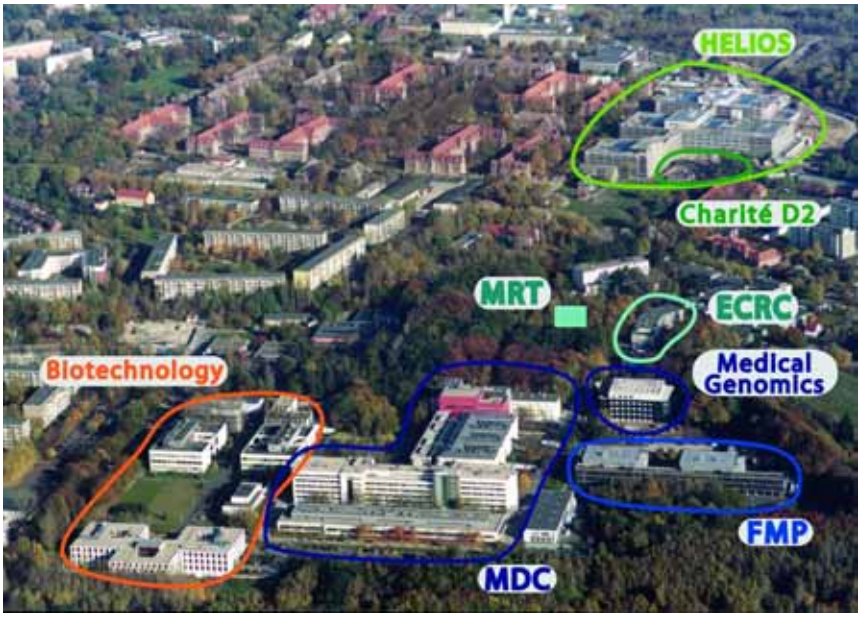
Milestones to be reached in the ECRC – Translational Research Center

The general mission of the ECRC is to use molecular approaches in order to improve the diagnosis and treatments for the most prevalent human diseases, namely cardiovascular disease, cancer, and neurological disorders. In the first five

Meilensteine der Forschung am ECRC

Die übergreifende Aufgabe des ECRC ist die Nutzung von Erkenntnissen aus der Molekularbiologie für die Verbesserung von Diagnose und Behandlung der häufigsten Krankheiten, insbesondere Herz- Kreislauferkrankungen, Krebs und neurodegenerative Erkrankungen. In den ersten 5 Jahren wird der Schwerpunkt auf der Entwicklung neuer diagnostischer Verfahren, wie z. B. nicht-invasiver Bildgebungsverfahren, liegen. Inwieweit Magnet-Resonanz-Tomographie (MRT) zur Identifizierung von Patienten mit einem erhöhten Risiko für Kardiomypathie beitragen, oder inwieweit eine nicht-invasive Darstellung des Gehirns helfen kann, das Ausmaß der Zerstörung nach einem Schlaganfall zu bestimmen, sind Beispiele der Fragestellungen, die im ECRC bearbeitet werden sollen. In Tiermodellen können bereits eine Vielzahl solcher biologischer Parameter gemessen werden. Die Anwendung dieser Methoden für Untersuchungen beim Menschen verfügbar zu machen, ist somit ein Schwerpunkt der Arbeit im ECRC. Verschiedene Bildgebungsverfahren sind notwendig, um einzelne Zellen im Organismus sichtbar zu machen und deren Weg im Organismus zu verfolgen. Diese Methoden ermöglichen z. B. das Studium der Funktion von Stammzellen im Gehirn, der Wanderung von Immunzellen im Körper sowie der Metastasierung von Tumoren. Langfristiges Ziel ist die Generierung und Nutzung neuer kleinmolekularer Substanzen, Antikörper, Impfstoffe oder modifizierter Zellen für verbesserte Therapien. Bereits bekannte kleinmolekulare Substanzen können im ECRC verbessert und weiterentwickelt werden. Bildgebungsverfahren sind wiederum notwendig, um den Erfolg und die Effizienz therapeutischer Substanzen zu bewerten, zunächst in Modellorganismen und letztendlich im Patienten. Die Entwicklung neuer Medikamente und Behandlungsmethoden für präklinische Studien, klinische Studien der Phase I und II, stehen im Mittelpunkt der Forschung des ECRC. Die Ent-

Luftbild des Campus Berlin-Buch, Darstellung des zukünftigen Standortes des ECRC, einschließlich der MRT-Einheit.
Photo / Copyright: Luftbild Flugservice Strausberg 2003
Aerial photo of the Campus Berlin-Buch showing the future location of the ECRC, including the MRT Unit.
Photo / Copyright: Luftbild Flugservice Strausberg 2003



years, we will focus on developing new diagnostic tools, like non-invasive imaging procedures. For instance, we will determine whether cardiac MRI is useful for identifying patients at risk for cardiomyopathy. Similarly, noninvasive brain imaging can identify, for instance, the extent of brain damage after stroke, and biological parameters that can be assessed currently in animal models can increasingly also be evaluated in humans. To follow the trafficking of cells in the organisms, another use of imaging technology, will enable the study of brain stem cell function, immune cell migration, and tumor metastasis. Our long-term goals entail the generation and use of tailored, small molecular weight compounds, antibodies, vaccines or modified cells in therapy. Imaging procedures will again be crucial to assess the efficacy of such therapeutic agents, first in model organisms and subsequently in patients. Initially, "lead" compounds developed elsewhere may be used and improved. However, subsequently we will develop new compounds and treatment procedures for preclinical, phase 1, and phase 2 clinical trials. Our objective in the ECRC will be to make available several compounds or treatment protocols for the three most prevalent human diseases, cardiovascular, cancer, and neurological diseases. Rational drug design requires collaboration of chemists, structural biologists, as well as cell biologists. Furthermore, preclinical and clinical studies require clinical pharmacologists and toxicologists. This expertise is available on the campus in Berlin Buch and/or at the Charité and will be available to ECRC investigators.

The MDC Berlin-Buch and the Helmholtz Association

The Helmholtz Association

The Helmholtz Association is the largest scientific organization in Germany with its 15 research centers and an annual budget of around 2.1 billion Euros. The 24,000 employees of the Helmholtz Association work in six research sectors. Ten Helmholtz centers work in the field of health research. The four largest among them are the DKFZ Cancer Research Center, Heidelberg, the GSF Research Center for the Environment and Health GmbH, the GBF Society for Biotechnological Research GmbH, and the MDC. The health research centers work in various areas of medical science, in particular in the exploration of the basics of biology, clinical applications, and general measures for the promotion of health (Public Health Research).

In September 2001, the Helmholtz Association was reorganized and the new instrument of program-oriented funding (PROF) was introduced. The aim of the program-oriented funding is the provision of resources for the six research sectors, in which programs are defined as regards to subject matter, which are implemented as long-term research objectives on the basis of cooperation and competition according to external evaluation. The Helmholtz Centers create their programs in association or individually and thus place themselves in competition within the Helmholtz Association. The programs and contributions of the individual centers are examined by international experts. The resulting recommendation for funding by the Helmholtz Association Senate is the basis for the financing of the centers by the grant providers. Seven programs have been defined within the research sector health. The MDC

wicklung bioaktiver Substanzen erfordert die Zusammenarbeit von Chemikern, Strukturbiologen und Biologen. Präklinische Studien und klinische Studien erfordern Pharmakologen und Toxikologen. Diese Expertise steht auf dem Campus Berlin-Buch sowie in der Charité und damit den Wissenschaftlern am ECRC zur Verfügung.

Das MDC Berlin-Buch und die Helmholtz-Gemeinschaft

Die Helmholtz-Gemeinschaft

Die Helmholtz-Gemeinschaft ist mit ihren 15 Forschungszentren und einem Jahresbudget von rund 2,1 Milliarden € die größte Wissenschaftsorganisation Deutschlands. Die 24.000 Mitarbeiterinnen und Mitarbeiter der Helmholtz-Gemeinschaft arbeiten in sechs Forschungsbereichen. Zehn Helmholtz-Zentren arbeiten auf dem Gebiet der Gesundheit. Die vier größten unter ihnen sind das DKFZ Krebsforschungszentrum Heidelberg, das GSF Forschungszentrum für Umwelt und Gesundheit GmbH, die GBF Gesellschaft für Biotechnologische Forschung GmbH und das MDC. Die Zentren der Gesundheitsforschung arbeiten in verschiedenen Bereichen der medizinischen Wissenschaft, insbesondere in der Erforschung der biologischen Grundlagen, der klinischen Anwendung und der allgemeinen, die Gesundheit fördernden Maßnahmen (Public Health Research).

Im September 2001 wurde die Helmholtz-Gemeinschaft reformiert und das neue Instrument der programmorientierten Förderung eingeführt. Ziel dieser Förderung ist die Bereitstellung der Ressourcen für die sechs Forschungsbereiche, indem thematisch Programme definiert sind, die auf der Basis von Kooperation und Wettbewerb nach externer Evaluierung zu langfristigen Forschungszielen durchgeführt werden. Die Helmholtz-Zentren erstellen im Verbund oder einzeln ihre Programme in eigener Verantwortung und stellen sich damit einem Wettbewerb innerhalb der Helmholtz-Gemeinschaft. Die Programme und Beiträge der einzelnen Zentren werden von Experten aus dem In- und Ausland begutachtet. Die daraus resultierende Förderempfehlung des Senats der Helmholtz-Gemeinschaft ist Grundlage für die Finanzierung der Zentren durch die Zuwendungsgeber. Innerhalb des Forschungsbereiches Gesundheit sind sieben Programme definiert worden. Das MDC Berlin-Buch ist dabei an drei Programmen beteiligt: Erforschung von Herz-Kreislauf- und Stoffwechselerkrankungen, Krebsforschung sowie die Erforschung der Funktion und Dysfunktion des Nervensystems.

Die Programmorientierte Förderung

Im Zusammenhang mit der programmorientierten Förderung der Helmholtz-Gemeinschaft sind die Programme Krebsforschung (im Juli 2002 in Heidelberg), Funktion und Dysfunktion des Nervensystems (im Juli 2002 in Jülich) und Herz-Kreislauf- und Stoffwechselerkrankungen (im September 2002 in Berlin-Buch) von international besetzten Gutachtergremien evaluiert worden. Im Gegensatz zu den Evaluationen der Jahre 1996 bis 1998, wo einzelne Arbeitsgruppen begutachtet wurden, war die Begutachtung zur programmorientierten Förderung eine strategische Programmbegutachtung.

Berlin-Buch is involved in the following three research programs: Cardiovascular and Metabolic Diseases, Cancer Research, and Function and Dysfunction of the Nervous System.

Program-Oriented Funding

In connection with the program-orientated funding (PROF) of the Helmholtz Association, the Research Programs Cancer Research (July 2002 in Heidelberg), Function and Dysfunction of the Nervous System (July 2002 in Jülich) and Cardiovascular and Metabolic Diseases (September 2002 in Berlin-Buch) were evaluated by expert committees with internationally drawn members. In contrast to the evaluations of the years 1996 to 1998, where individual laboratory groups were examined, the examination in respect to PROF was a strategic program examination.

Laboratory for Medical Genome Research

Already before the Helmholtz Association evaluation occurred, the construction of a new building for medical genome research on the Berlin-Buch Campus, supported with considerable European funds European Fund for Regional Development *(EFRE)*, had been confirmed within the framework of the program-oriented funding by the grant providers. In the opinion of the experts, the surplus funding received within the framework of program-oriented funding to expand the MDC Berlin-Buch in the area of medical genome research is justified.

The construction of the Laboratory for Medical Genome Research is a joint project of the MDC Berlin-Buch and the Research Institute for Molecular Pharmacology (*FMP*) and, thus, 62.5% of the costs were financed by the European Funds for Regional Development *(EFRE)*. The new laboratory building was constructed at the end of the main axis of the Campus grounds in the direct vicinity of the FMP and the Walter-Friedrich House of the MDC. Construction will be completed by December 2005. The new building of the Laboratory for Medical Genome Research will serve to create the spatial preconditions necessary for investigating new questions in genome research.

The MDC Berlin-Buch and the Campus Berlin-Buch

BBB Management GmbH with Innovation and Founder Center

The BBB Management GmbH (BBB) was founded by the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch in 1995. The Institute for Molecular Pharmacology (*FMP*) as well as Schering AG are co-shareholders (20 percent each). The BBB acts as the development and coordination company for the entire research campus in Berlin-Buch, managing a commercial area of around 26,000 square meters. As such, it operates an Innovation and Founder Center *(IGZ)* for new biotechnology companies and is in charge of the real estate on the Campus. Here, small and medium-sized enterprises carry out research and production. In 2003, several enterprises, including three start-up companies, moved onto the Campus. Currently, around 45 companies are located on the Campus, of which around 35 are in the biotechnology

Das Arnold-Graffi-Haus des Biotechnologieparks auf dem Campus Berlin-Buch.
Photo: Uwe Eising / Copyright: MDC
The Arnold Graffi Building of the Biotechnology Park on the Campus Berlin-Buch.
Photo: Uwe Eising / Copyright: MDC

Labor für medizinische Genomforschung

Bereits vor dem Begutachtungsverfahren im Rahmen der programmorientierten Förderung war vonseiten der Zuwendungsgeber ein Neubau für die medizinische Genomforschung auf dem Campus Berlin-Buch mit erheblichen europäischen Mitteln (EFRE) bestätigt worden. Die Voten der Gutachter zu den Überzeichnungen im Rahmen der programmorientierten Förderung haben die Ausbauziele des MDC Berlin-Buch im Bereich der medizinischen Genomforschung unterstützt. Das Labor für Medizinische Genomforschung ist ein gemeinsamer Bau des MDC Berlin-Buch und des Forschungsinstituts für Molekulare Pharmakologie (FMP), dabei werden 62,5 Prozent der Kosten aus dem Europäischen Fonds für Regionalentwicklung (EFRE) finanziert. Der Laborneubau wurde am Ende der Hauptachse des Campusgeländes in direkter Nachbarschaft zum FMP und dem Walter-Friedrich-Haus des MDC errichtet. Das Gebäude wurde im Dezember 2005 fertig gestellt. Im Jahr 2006 werden mehrere Forschungsgruppen, die auch neu an das MDC/FMP berufen werden, ihre Arbeit in diesem Neubau aufnehmen. Dort wurden die räumlichen Kapazitäten ausgebaut und damit die Voraussetzungen dafür geschaffen, neue Fragestellungen in der Genomforschung zu bearbeiten.

Das MDC Berlin-Buch und der Campus-Berlin-Buch

Biotechnologiepark mit Innovations- und Gründerzentrum

Im direkten Umfeld von Grundlagenforschung und klinischer Praxis ist auf dem Campus Berlin-Buch seit Beginn der 1990er Jahre ein Technologiepark mit Innovations- und Gründerzentrum (IGZ) mit einer vermietbaren Fläche von rund 26.000 m² entstanden, in dem kleine und mittelständige Unternehmen forschen und produzieren. Gegenwärtig sind auf dem Campus rund 45 Unternehmen angesiedelt. Davon sind rund 35 Biotechnologieunternehmen, die verbleibenden Unternehmen sind in den Bereichen Support und Services vorwiegend für Biotech tätig. Die Firmen beschäftigen insgesamt circa 500 Mitarbeiter. Trotz der Ende 2000 einsetzenden

sector. The remaining firms are mainly active in the areas of biotechnical support and services. Together, the firms employ approximately 500 employees.

In 2003, the BBB Management GmbH opened a Laboratory and Bio Computer Science building constructed at a cost of around 16 million Euros and, simultaneously, celebrated the five-year existence of the IGZ. The new building has 8,000 square meters of commercial space and serves as the headquarters for various enterprises. The extension of the IGZ has been completed with this new building. Therefore, the Berlin-Buch Campus has one of the largest sector-specific centers for new businesses in Germany at its disposal.

Life Science Learning Laboratory

In the Life Science Learning Laboratory *(Gläsernes Labor)* visitors can independently carry out gene technology and cell biological experiments in authentic research laboratories and discuss their concrete applications in research, medicine, and biotechnology with scientists. Since 2001, the Life Science Learning Laboratory has participated in the "Long Night of Sciences" on the Berlin-Buch Campus with over 3,000 visitors per year visiting the Berlin-Buch campus. In 2003 alone, more than 7,100 visitors took advantage of the advanced training and continuing education classes including courses in gene technology, cell biology and protein analysis. In addition, in 2003 a new opportunity for laboratory workers to gain qualifications in molecular biology was offered jointly with the German authority for monitoring technical standards (TÜV Academy Berlin). In 2003, the BBB Management GmbH opened an information bureau within the Life Science Learning Laboratory building. It has a Berlin-Buch Campus exhibition and is the starting point for guided tours of the Campus.

InnoRegio-Initiative Berlin-Buch

InnoRegio is a funding program that was established in 1999 by the German Federal Ministry of Research *(BMBF)*. The InnoRegio program aims to encourage and support development and investment in areas of science, education, and business in order to make the specific region or location more commercially attractive and competitive.

The InnoRegio Project has given rise to a clinical research network in Buch. The main focus of the initiative is to interlink the Berlin-Buch research institutes, clinics, and companies in regard to developing novel forms of therapeutic approaches, biomedical technologies, and clinical applications. The first projects involve the fields of molecular tumor diagnosis, genetic epidemiology based on twin studies, the preclinical and clinical testing and development of a new lipid-based administration form (drug delivery system) for the anticancer drug, Taxol, as well as protein- and active ingredient-screening. The funding for the InnoRegio Berlin-Buch up until 2005 amounts to 5.2 million Euro.

und noch anhaltenden Konsolidierungsphase in der Biotechnologie gelang es, insbesondere 2004/05 mehr als 10 neue Unternehmen, darunter auch mehrere Neugründungen auf dem Campus anzusiedeln.

2003 hat die BBB Management GmbH Campus Berlin-Buch, die Betreiber- und Entwicklungsgesellschaft des Campus, ein mit rund 16 Millionen Euro errichtetes Labor- und Bioinformatikgebäude eröffnet und damit gleichzeitig das fünfjährige Bestehen des IGZ gefeiert. Damit verfügt der Campus über eines der größten branchenspezifischen Gründerzentren in Deutschland. Allein die BBB GmbH hat seit ihrer Gründung rund 60 Mill. € in den Ausbau und die Modernisierung des Campus, seiner Gemeinschaftseinrichtungen und seines Biotechnologieparks investiert. In der Förderung sind Mittel der Gemeinschaftsaufgabe zur Verbesserung der regionalen Wirtschaftsstruktur und des Europäischen Fonds für Regionale Entwicklung enthalten. Zur Sicherstellung der Nachhaltigkeit der Campusförderung hat sich der Campus über die Campusgesellschaft BBB GmbH auch intensiv in die weitere Entwicklung seines Umfeldes, des Stadtteils Berlin-Buch, zu einer Gesundheitsregion eingebracht. Die BBB Management GmbH Campus Berlin-Buch wurde vom Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch gegründet. Mitgesellschafter sind das Forschungsinstitut für Molekulare Pharmakologie (20%) sowie die Schering AG (20%).

Gläsernes Labor

Im Gläsernen Labor können Besucher in authentischen Forschungslaboren gentechnische und zellbiologische Experimente durchführen und mit Wissenschaftlern über konkrete Anwendungen in Forschung, Medizin und Biotechnologie diskutieren. Allein im Jahre 2004 nutzten mehr als 7.000 Besucher – vor allem Schulklassen – die umfassenden Angebote zur Fort- und Weiterbildung. Zusätzlich zu den bisherigen Kursen für die Oberstufe werden seit 2004 auch Kurse für die Sekundarstufe I angeboten. Dies wurde 2004 bereits von 41 Schulklassen (über 900 Schülern) und 2005 von über 100 Klassen genutzt. Seit 2003 werden regelmäßig zweimal im Jahr gemeinsam mit der TÜV Akademie Berlin Qualifizierungslehrgänge für Laborkräfte angeboten – u.a. ein zehnwöchiger zertifizierter Weiterbildungskurs zur „Fachkraft für Molekularbiologie".

Seit dem Jahre 2001 konzipiert und realisiert die vom Gläsernen Labor der BBB GmbH koordinierte Öffentlichkeitsarbeit zusammen mit allen Einrichtungen des Campus die „Lange Nacht der Wissenschaften" auf dem Campus Berlin-Buch. Über 3000 Besucher haben diese Veranstaltung pro Jahr auf dem Campus besucht.

InnoRegio Berlin-Buch

InnoRegio wurde 1999 vom Bundesministerium für Bildung und Forschung (BMBF) gestartet. Ziel des InnoRegio-Programms ist die Verstärkung von Entwicklung und Investition in den Bereichen Wissenschaft, Bildung und Wirtschaft, um Wertschöpfung und Wettbewerbsfähigkeit in bestimmten Regionen zu steigern.

Im Bereich klinische Forschung und Entwicklung ist mit dem InnoRegio-Projekt ein Bucher Netzwerk entstanden. Kernanliegen ist dabei die Vernetzung der in Berlin-Buch ansässigen

Institute for Molecular Pharmacology (*FMP*)

The Institute for Molecular Pharmacology (*FMP*) is engaged in basic research in the identification and characterization of biological macromolecules as drug targets. Through the close spatial proximity to the MDC, the already existing collaboration between the two research centers has 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 well as the development of strategies for their pharmacological influence.

The close connection between the two research establishments extends into the organizational level. Thus, large equipment is shared and jointly operated. 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.

Forschungsinstitute, Kliniken und Unternehmen im Hinblick auf die Entwicklung neuartiger therapeutischer Ansätze, biomedizinischer Technologien und klinischer Anwendungen. Erste Projekte umfassen die Bereiche der molekularen Tumordiagnostik, der genetischen Epidemiologie auf der Basis von Zwillingsstudien, die präklinische und klinische Prüfung und Entwicklung von neuen, lipidbasierten Darreichungsform (drug delivery system) für das Krebsmedikament Taxol, sowie das Protein- und Wirkstoffscreening. Insgesamt wurde InnoRegio Berlin-Buch bis zum Jahr 2005 mit 5,2 Mill. € gefördert.

Forschungsinstitut für Molekulare Pharmakologie (FMP)

Das FMP betreibt Forschung auf dem Gebiet der Molekularen Pharmakologie und forscht im Vorfeld der Entwicklung von Arzneimitteln. Es verfolgt dazu einen interdisziplinären Forschungsansatz, der zur Zusammenführung von Zellulärer Signaltransduktion/Molekularer Genetik, Strukturbiologie und Chemischer Biologie geführt hat. Kennzeichnend für die wissenschaftliche Arbeit am FMP ist die enge Verknüpfung von Chemie und Biologie.

Durch die räumliche Nähe ist die Zusammenarbeit zwischen MDC und FMP über die zurückliegenden Jahre kontinuierlich ausgebaut worden. Die Forschungskonzepte des MDC und des FMP ergänzen sich: Während sich die molekularmedizinische Forschung am MDC besonders Erkrankungen oder klinischen Symptomen und deren molekularen Erklärungen widmet, versucht das FMP, die Struktur, Funktionen und Interaktionen von Proteinen aufzuklären und neue Konzepte zu ihrer pharmakologischen Beeinflussung zu entwickeln.

Auch auf der organisatorischen Ebene gibt es eine enge Zusammenarbeit. So werden Großgeräte gemeinsam genutzt und betrieben. Gastwissenschaftlerverträge ermöglichen Wissenschaftlern der einen Einrichtung die Ausstattung einer gastgebenden Arbeitsgruppe in der anderen Einrichtung zu nutzen. Beide Institute entsenden Vertreter in wichtige Gremien der jeweils anderen Einrichtung. Die Planung aufwendiger und langfristiger Forschungsprojekte sowie die Berufung von leitenden Wissenschaftlern erfolgen in gegenseitiger Absprache. Darüber hinaus gestalten und finanzieren MDC und FMP gemeinsam Veranstaltungen für Doktoranden.

Organizational Structure

The Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch is a foundation that was established under the laws of Berlin whose purpose is to conduct medical research at the molecular and cellular levels and pursue its clinical application and realization. The Foundation's governing bodies are the Board of Trustees, the Scientific Committee, and the Executive Board.

Board of Trustees

The Board of Trustees is the supervisory body of the MDC and monitors the legality, effectiveness, and economic efficiency of the operation of the *Stiftungsgesetz* (German Foundation Act). It decides upon the Foundation's general research targets and important research policy and financial matters.

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Prof. Dr. Hans Jürgen Prömel, Vice-President of the Humboldt University Berlin

Organisationsstruktur

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 Umsetzung zu betreiben. Organe der Stiftung sind das Kuratorium und dessen Wissenschaftlicher Ausschuss sowie der Stiftungsvorstand.

Das Kuratorium

Das Kuratorium ist das Aufsichtsgremium des MDC und überwacht die Rechtmäßigkeit, Zweckmäßigkeit und Wirtschaftlichkeit der Führung des Stiftungsgesetzes. Es entscheidet über die allgemeinen Forschungsziele und wichtigen forschungspolitischen und finanziellen Angelegenheiten der Stiftung.

Mitglieder des Kuratoriums

Ministerialdirektor Reinhard Junker, Bundesministerium für Bildung und Forschung (BMBF), Berlin (Vorsitz)
Senatsdirigent Wolfgang Eckey, Senatsverwaltung für Wissenschaft, Forschung und Kultur, Berlin (stellv. Vorsitz)
Prof. Dr. Günter Breithardt, Universität Münster*
Prof. Dr. Kurt von Figura, Universität Göttingen* (bis Ende 2005)
Prof. Dr. Ulrich Frei, Charité - Universitätsmedizin Berlin
Prof. Dr. Annette Grüters-Kieslich, Charité - Universitätsmedizin Berlin*
Prof. Dr. Wieland Huttner, Max-Planck-Institut für molekulare Zellbiologie und Genetik, Dresden*
Prof. Dr. Reinhard Jahn, Max-Planck-Institut für biophysikalische Chemie, Göttingen*
Ministerialrat Oskar-Peter Kaye, Bundesministerium für Bildung und Forschung, Bonn (bis Sept. 2005)
Prof. Dr. Dieter Lenzen, Freie Universität Berlin
Prof. Dr. Maria Leptin, Institut für Genetik der Universität zu Köln*
Prof. Dr. Gary R. Lewin, MDC Berlin-Buch

Prof. Dr. Annemarie Poustka, German Cancer Research Center Heidelberg*
Staatssekr. Dr. Hermann Schulte-Sasse, Senate Administration For Health, Social Services and Consumer Protection, Berlin
Department Head Dr. Albert Statz, Federal Ministry of Health, Bonn
Prof. Dr. Axel Ullrich, Max Planck Institute for Biochemistry, Martinsried*
Senior Government Officer Hans-Ulrich Weber, Federal Ministry of Finances, Berlin
Dr. Ulrike Ziebold, MDC Berlin-Buch

* Member of the Scientific Committee

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 the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch through scientific assessment. Together with the scientific members of the Board of Trustees, up to eight external specialists sit on the Scientific Committee.

Members of the Scientific Committee
Prof. Dr. Wieland Huttner, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden (Chair)*
Prof. Dr. Kurt von Figura, University of Göttingen* (until end of 2005)
Prof. Dr. Rudi Balling, German Research Centre for Biotechnology, GBF, Braunschweig
Prof. Dr. Günter Breithardt, University of Münster*
Prof. Dr. Roger Goody, Max Planck Institute for Molecular Physiology, Dortmund
Prof. Dr. Annette Grüters-Kieslich, Charité University Medicine Berlin*

Preisträger von „Jugend forscht“ im Labor von Prof. Walter Birchmeier im Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch.
Photo: Thomas Oberländer / Copyright: Helios Klinikum Berlin-Buch
Recipients of the “Youth in Research” (Jugend forscht) award in the laboratory of Prof. Walter Birchmeier at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch.
Photo: Thomas Oberländer / Copyright: Helios Klinikum Berlin-Buch.

Prof. Dr. Hans Jürgen Prömel, Vizepräsident der Humboldt-Universität zu Berlin
Prof. Dr. Annemarie Poustka, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg*
Staatssekretär Dr. Hermann Schulte-Sasse, Senatsverwaltung für Gesundheit, Soziales und Verbraucherschutz, Berlin
Ministerialrat Dr. Albert Statz, Bundesministerium für Gesundheit, Bonn
Prof. Dr. Axel Ullrich, Max-Planck-Institut für Biochemie, Martinsried*
Regierungsdirektor Hans-Ulrich Weber, Bundesministerium der Finanzen, Berlin
Dr. Ulrike Ziebold, MDC Berlin-Buch

* zugleich Mitglieder des Wissenschaftlichen Ausschusses

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 acht externe Fachwissenschaftler an.

Mitglieder des Wissenschaftlichen Ausschusses
Prof. Dr. Wieland Huttner, Max-Planck-Institut für molekulare Zellbiologie und Genetik, Dresden (Vorsitz)*
Prof. Dr. Kurt von Figura, Universität Göttingen* (bis Ende 2005)
Prof. Dr. Rudi Balling, Gesellschaft für Biotechnologische Forschung (GBF), Braunschweig
Prof. Dr. Günter Breithardt, Universität Münster*
Prof. Dr. Roger Goody, Max-Planck-Institut für molekulare Physiologie, Dortmund
Prof. Dr. Annette Grüters-Kieslich, Charité - Universitätsmedizin Berlin*
Prof. Dr. Christoph Huber, Universität Mainz
Prof. Dr. Reinhard Jahn, Max-Planck-Institut für biophysikalische Chemie, Göttingen (Stellv. Vorsitz)*
Prof. Dr. Maria Leptin, Institut für Genetik der Universität zu Köln*
Prof. Dr. Thomas Meitinger, Forschungszentrum für Umwelt und Gesundheit (GSF), Neuherberg
Prof. Dr. Leena Peltonen, Universität Helsinki, Finnland
Prof. Dr. Annemarie Poustka, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg*
Prof. Sir George K. Radda, Universität Oxford, Großbritannien
Prof. Dr. Axel Ullrich, Max-Planck-Institut für Biochemie, Martinsried*

* zugleich Mitglieder des Kuratoriums

Prof. Dr. Christoph Huber, University of Mainz
Prof. Dr. Reinhard Jahn, Max Planck Institute for Biophysical Chemistry, Göttingen (Vice-Chair)*
Prof. Dr. Maria Leptin, Institute for Genetics, University of Cologne*
Prof. Dr. Thomas Meitinger, GSF - National Research Center for Environment and Health, Neuherberg
Prof. Dr. Leena Peltonen, University of Helsinki, Finland
Prof. Dr. Annemarie Poustka, German Cancer Research Center, Heidelberg*
Prof. Sir George K. Radda, University of Oxford, Great Britain
Prof. Dr. Axel Ullrich, Max Planck Institute for Biochemistry, Martinsried*

* Member of the Board of Trustees

Executive Board

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.

Scientific Council

The Scientific Council advises the Executive Board in matters of fundamental scientific importance.

Members of the Scientific Council:
Prof. Dr. Thomas Blankenstein
Dr. Kurt Bommert (until Summer 2005)
Dr. Iduna Fichtner
Dr. Hannelore Haase
Prof. Dr. Udo Heinemann
Dr. Uta Höpken
Prof. Dr. Helmut Kettenmann
Prof. Dr. Gary Lewin
Dr. Martin Lipp (Chair)
Prof. Dr. Friedrich Luft
Dr. Margret Irmgardt Moré
Dr. Thomas Müller
Prof. Dr. Claus Scheidereit
Prof. Dr. Peter Schlag
Dr. Katrin Stade
Dr. Ruth Schmidt-Ullrich
Prof. Dr. Wolfgang Uckert (Vice-Chair)
Dr. Gerd Wallukat

Das Max-Delbrück-Haus (rechts) mit Flachbau. Es ist eines von 3 großen Laborgebäuden des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch.
Photo: Uwe Eising/Copyright: MDC
The Max Delbrück building (right) with building extension - one of the 3 main laboratory buildings of the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch.
Photo: Uwe Eising/Copyright: MDC

Der Stiftungsvorstand

Der Stiftungsvorstand leitet das Institut und besteht aus einem wissenschaftlichen Mitglied, Prof. Walter Birchmeier, und einem administrativen Mitglied, Dr. Stefan Schwartze. Vorsitzender des Stiftungsvorstands ist Prof. Dr. Walter Birchmeier.

Der Wissenschaftliche Rat

Der Wissenschaftliche Rat berät den Stiftungsvorstand in Angelegenheiten von grundsätzlicher wissenschaftlicher Bedeutung.

Mitglieder des Wissenschaftlichen Rates
Prof. Dr. Thomas Blankenstein
Dr. Kurt Bommert (bis Sommer 2005)
Dr. Iduna Fichtner
Dr. Hannelore Haase
Prof. Dr. Udo Heinemann
Dr. Uta Höpken
Prof. Dr. Helmut Kettenmann
Prof. Dr. Gary Lewin
Dr. Martin Lipp (Vorsitz)
Prof. Dr. Friedrich Luft
Dr. Margret Irmgardt Moré
Dr. Thomas Müller
Prof. Dr. Claus Scheidereit
Prof. Dr. Peter Schlag
Dr. Katrin Stade
Dr. Ruth Schmidt-Ullrich
Prof. Dr. Wolfgang Uckert (stellv. Vorsitz)
Dr. Gerd Wallukat

Staff Council

The Staff Council is involved in decisions of the MDC that concern personnel and staff welfare matters.

Members of the Staff Council
Marion Bimmler (Chair)
Lutz Else (Vice-Chair)
Dr. Bettina Erdmann
Dagmar Gerhard
Frank-Peter Kirsch
Dr. Peter Konzer
Bernd Lemke (Vice-Chair)
Dr. Thomas Müller
Martin Pflaume
Jana Richter
Christel Westen

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. Katrin Stade serves as the MDC women's representative.

Der Personalrat

Der Personalrat ist an solchen Entscheidungen des MDC Berlin-Buch beteiligt, welche die personellen und sozialen Belange der Beschäftigten betreffen.

Mitglieder des Personalrates
Marion Bimmler (Vorsitz)
Lutz Else (stellv. Vorsitz)
Dr. Bettina Erdmann
Dagmar Gerhard
Frank-Peter Kirsch
Dr. Peter Konzer
Bernd Lemke (stellv. Vorsitz)
Dr. Thomas Müller
Martin Pflaume
Jana Richter
Christel Westen

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. Katrin Stade die Funktion der Frauenvertreterin am MDC wahr.

Konzentriert: Preisträger von „Jugend forscht“ bei einer Informationsveranstaltung des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch.
Photo: Thomas Oberländer/Copyright: Helios Klinikum Berlin-Buch
Concentrated: Recipients of the “Youth in Research” (Jugend forscht) award listen to a seminar at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch.
Photo: Thomas Oberländer/Copyright: Helios Clinic Berlin-Buch

Facts and Figures

Fakten und Kennzahlen

Financing

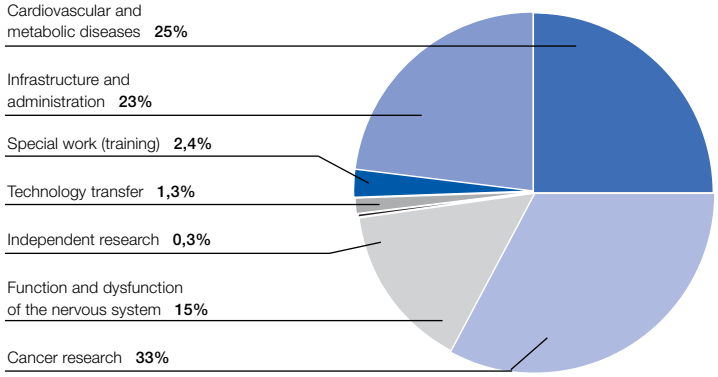
During 2004, the MDC had € 47,247,000 available in the framework of program-oriented research for financing research. In addition, third-party funds and research commissions in the amount of € 11,621,000 were acquired.

Finanzierung

Für das Jahr 2004 standen dem MDC 47.247.000 € im Rahmen der Programmorientierten Förderung zur Finanzierung der Forschungsarbeiten zur Verfügung. Zusätzlich konnten Drittmittel und Forschungsaufträge i. H. v. 11.621.000 € eingeworben werden.

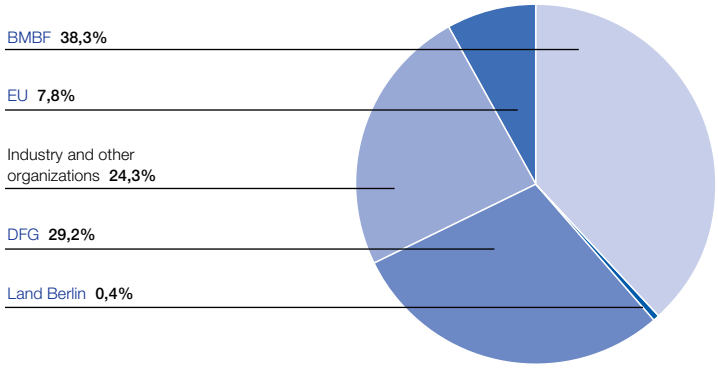
Costs of research programs in 2004 (in thousands of €)
Kosten der Forschungsprogramme 2004 in T€

Programs / Categories Programme / Kategorien	Costs Kosten
Cardiovascular and metabolic diseases Herz-Kreislauf- und Stoffwechselerkrankungen	11,705
Cancer research Krebsforschung	15,564
Function and dysfunction of the nervous system Funktion und Dysfunktion des Nervensystems	7,148
Independent research Programmungebundene Forschung	113
Infrastructure and administration Infrastruktur und Verwaltung	10,947
Technology transfer Technologietransfer	633
Special work (training) Sonderaufgaben (Ausbildung)	1,137
Total Gesamt	47,247



Extramural funding in 2004 (in thousands of €)
Drittmittelfinanzierung 2004 in T€

Extramural funds Drittmittelgeber	**Amounts** Drittmittelerlöse
Federal Ministry of Education and Research Bundesministerium für Bildung und Forschung (BMBF)	**4.450**
German Research Foundation Deutsche Forschungsgemeinschaft (DFG)	**3.396**
Industry and other organizations Industrie und sonstige Organisationen	**2.829**
EU EU	**906**
State of Berlin Land Berlin	**40**
Total Gesamt	**11.621**



Personnel

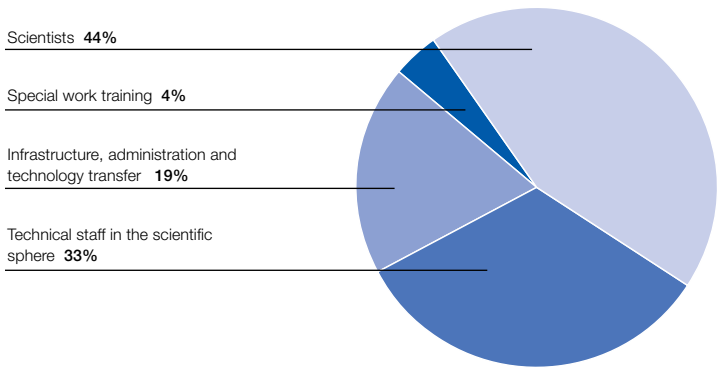
At present, the MDC has 707 employees (as of July 2005). Of these, 30% are financed via third-party funds. Of the scientific staff, 47% are financed via third-party funds. Sixty per cent of all MDC employees have limited period contracts of employment; this figure is 79% in the scientific sector.

Personal

Im MDC sind derzeit 707 Mitarbeiter beschäftigt (Stand: Juli 2005). Von diesen Personen sind 30% über Drittmittel finanziert. Bei den wissenschaftlichen Mitarbeitern beträgt der Anteil der aus Drittmitteln finanzierten Personen 47%. Der Gesamtanteil der befristet Beschäftigten am MDC liegt bei 60%. Im wissenschaftlichen Bereich sind es 79%.

Personnel structure 2004 in person years (PY)
Personalstruktur 2004 in Personenjahren (PJ)

Categories Kategorien	**PY PJ** **Basic financing** Grundfinanzierung	**PY PJ** **third-party financing** Drittmittel
Scientists Wissenschaftler	**161,0**	**147,3**
Technical staff in the scientific sphere Technische Angestellte im wissenschaftlichen Bereich	**163,5**	**67,6**
Infrastructure, administration and technology transfer Infrastruktur, Verwaltung und Technologietransfer	**129,4**	**8,1**
Special work training Sonderaufgaben (Ausbildung)	**28,8**	
Total Gesamt	**482,7**	**223,0**



Involvement of MDC personnel in national and international research programmes

Special research fields of the German Research Foundation (DFG)

SFB 366	Cellular signal detection and realization
SFB 507	The importance of non-neuronal cells with neurological disorders
SFB 515	Mechanisms of development- and experience-dependent plasticity of the nervous system
SFB 577	Molecular foundations of the clinical variability of monogenic diseases
SFB 594	Molecular machines in protein folding and protein transport
SFB 618	Theoretical biology: Robustness, modularity and evolutionary design of living systems
SFB 633	Induction and modulation of T cell-imparted immunoreactions in the gastrointestinal tract
SFB 650	Cellular approaches to the suppression of undesirable immunoreactions - from bench to bedside
SFB 665	Development disorders in the nervous system
SFB 6040	Transregio 19: Inflammatory myocardiopathy - molecular pathogen and treatment

National Genome Research Network (NGFN-2) Projects

Genome network cardiovascular diseases; TP1: Comparative genomics of left ventricular hypertrophy and dysfunction in hypertension
Genome network cardiovascular diseases; TP2: Functional genomics of cardiac damage in hypertension
Genome network CancerNet: Organ specificity of colorectal cancer metastasis
Genome network NeuroNet: Moleculargenetic identification of disposing gene configurations with genetically determined epilepsies
Genome network cardiovascular diseases: Prevalence of titin mutations and identification of new disease genes in patients with familial DCM
Systematic-methodological platform “DNA”, location MDC, Berlin, TP5: SNP Map Rat
Systematic-methodological platform “DNA”, TP12.1(2): SNP Map - National geno-typing platform
Systematic-methodological platform “GEM”: Genome-wide coupling analysis and association studies with then 10K and 100K Chips
Systematic-methodological platform “Protein”: Verification and identification of protein-protein interactions and systematic analysis of target proteins by means of X-ray structural analysis, TP 7 - Structure determination
Systematic-methodological platform “Protein”: Verification and identification of protein-protein interactions and systematic analysis of target proteins by means of X-ray structural analysis, TP 20 - Project management
Systematic-methodological platform “Protein”: Verification and identification of protein-protein interactions and systematic analysis of target proteins by means of X-ray structural analysis, TP 8 Yeast two-hybrid protein interaction networks
Systematic-methodological platform “Protein”: Verification and identification of protein-protein interactions and systematic analysis of target proteins by means of X-ray structural analysis, TP 1.1.: Subcloning of ORFs
Determining the genes on which the Williams-Beuren syndrome is based through the generation of an allelic series of mutations with the help of innovative transposon approaches

EU programmes

Transgenic models for cardiovascular diseases (Marie Curie training sites)
Multi-organismic approach to study normal and aberrant muscle development, function and repair (MYORES)
Translational and functional onco-genomics: from cancer-oriented genomic screenings to new diagnostic tools and improved cancer treatment (Transfog)
High throughput development of drugs for immunotherapy of autoimmune diseases (Drugs for Therapy)
Structural proteomics in Europe (SPINE)
An SNP and haplotype map for the rat (STAR)
Functional genomics in engineered ES cells (FunGenES)
Function of C/EBP beta in bone development and bone tumourigenesis (Marie Curie Fellowship)
Alternative transcript diversity project (ATD)
Role of transcription factor NF-kB in heart failure and arteriosclerosis (Host Fellowships)
Identification of Novel Target Genes for Cancer Therapy (INTACT)
Abnormal proteins in the pathogenesis of neurodegenerative disorders (APOPIS)
European integrated project on spinocerebellar ataxias (EUROSCA): Pathogenesis, genetics, animal models and therapy
European Renal Genome Project (EuReGene)
A systematic approach to find new cancer molecules: an enhancer-trap screen to identify genes required for proliferation and differentiation in murine stem cells (ES-TRAP)

Beteiligungen von MDC-Wissenschaftlern an nationalen und internationalen Forschungsprogrammen

Sonderforschungsbereiche der DFG

SFB 366	Zelluläre Signalerkennung und -umsetzung
SFB 507	Die Bedeutung nicht-neuronaler Zellen bei neurologischen Erkrankungen
SFB 515	Mechanismen entwicklungs- und erfahrungsabhängiger Plastizität des Nervensystems
SFB 577	Molekulare Grundlagen klinischer Variabilität monogen bedingter Krankheiten
SFB 594	Molekulare Maschinen in Proteinfaltung und Proteintransport
SFB 618	Theoretische Biologie: Robustheit, Modularität und evolutionäres Design lebender Systeme
SFB 633	Induktion und Modulation T-zellvermittelter Immunreaktionen im Gastrointestinaltrakt
SFB 650	Zelluläre Ansätze zur Suppression unerwünschter Immunreaktionen – from bench to bedside
SFB 665	Entwicklungsstörungen im Nervensystem
SFB 6040	Transregio 19: Inflammatonische Kardiomyopathie – Molekulare Pathogenes und Therapie

Projekte des Nationalen Genomforschungsnetzes (NGFN-2)

Genomnetz Herz-Kreislauf; TP1: Comparative genomics of left ventricular hypertrophy and dysfunction in hypertension
Genomnetz Herz-Kreislauf; TP2: Functional genomics of cardiac damage in hypertension
Genomnetz CancerNet: Organ Specifity of Colorectal Cancer Metastasis
Genomnetz NeuroNet: Molekulargenetische Identifizierung von disponierenden Genkonfigurationen bei genetisch determinierten Epilepsien
Genomnetz Herz-Kreislauf: Prävalenz von Titin-Mutationen und Identifizierung neuer Krankheitsgene in Patienten mit Familiärer Dilativer Kardiomyopathie
Systematisch-Methodische Plattform „DNA“, Standort MDC, Berlin, TP5: SNP Map Rat
Systematisch-Methodische Plattform „DNA“, TP12.1(2): SNP Map – Nationale Genotypisierungsplattform
Systematisch-methodische Plattform „GEM“: Genomweite Kopplungsanalyse und Assoziationsstudien mit den 10K und 100K Chips
Systematisch-methodische Plattform „Protein“: Vertifikation und Identifikation von Protein-Protein Interaktionen und systematische Analyse von Targetproteinen mittels Röntgenstrukturanalyse, TP 7 – Structure determination
Systematisch-methodische Plattform „Protein“: Vertifikation und Identifikation von Protein-Protein Interaktionen und systematische Analyse von Targetproteinen mittels Röntgenstrukturanalyse, TP 20 – Project 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 interaction networks
Systematisch-methodische Plattform „Protein“: Vertifikation und Identifikation von Protein-Protein Interaktionen und systematische Analyse von Targetproteinen mittels Röntgenstrukturanalyse, TP 1.1.: Subcloning of ORFs
Bestimmung der Gene, die dem Williams-Beuren-Syndrom zugrunde liegen durch generieren einer allelischen Serie von Mutationen mit Hilfe von neuartigen Transposon Ansätzen

EU-Programme

Transgenic models for cardiovascular diseases (Marie Curie training sites)
Multi-organismic Approach to study Normal and Aberrant Muscle Development, Function and Repair (MYORES)
Translational and Functional Onco-Genomics: from cancer-oriented genomic screenings to new diagnostic tools and improved cancer treatment (Transfog)
High Throughput development of drugs for immunotheraphy of autoimmune dieseases (Drugs for Therapy)
Structural proteomics in Europe (SPINE)
A SNP and haplotype map for the rat (STAR)
Functional Genomics in Engineered ES cells (FunGenES)
Function of C/EBP beta in bone development and bone tumourigenesis (Marie Curie Fellowship)
The Alternative Transcript Diversity Project (ATD)
Role of transcription factor NF-kB in heart failure and arteriosclerosis (Host Fellowships)
Identification of Novel Target Genes for Cancer Therapy (INTACT)
Abnormal proteins in the pathogenesis of neurodegenerative disorders (APOPIS)
European integrated project on spinocerebellar ataxias (EUROSCA): Pathogenesis, genetics,animal models and therapy
European Renal Genome Project (EuReGene)
A systematic approach to find new cancer molecules: an anhancer-trap screen to identify genes required for proliferation and differentiation in murine stem cells (ES-TRAP)

Das Labor für Medizinische Genomforschung im Herbst 2005 kurz vor der Fertigstellung. Bauherren sind das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch und das Forschungsinstitut für Molekulare Pharmakologie (FMP).
Photo: Ralf Streckwall/Copyright: MDC
The Laboratory for Medical Genome Research in the Fall of 2005 just prior to completion. The building was jointly constructed by the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch and the Institute for Molecular Pharmacology (*FMP*).
Photo: Ralf Streckwall/Copyright: MDC

Graduate Schools of the German Research Foundation (DFG)

GK 120:	Signal chains in living systems
GK 238:	Compensatory mechanisms in the nervous system: Application of imaging techniques
GK 268:	Dynamics and evolution of cellular and macromolecular processes
GK 429:	Neuropsychology and psychiatry of aging
GK 865:	Vascular regulation mechanisms
GK 1123:	Cellular mechanisms of learning and memory formation as well as memory consolidation
GK:	Biophysical investigations of protein interactions (assessed positively)
GK:	International Max Planck Research School: The life course: Evolutionary and ontogenetic dynamics (LIFE)

Graduiertenkollegs der DFG

GK 120:	Signal Chains in living Systems
GK 238:	Compensatory Mechanisms in the Nervous System: Application of Imaging Techniques
GK 268:	Dynamics and Evolution of cellular and macromolecular Processes
GK 429:	Neuropsychology and Psychiatry of Aging
GK 865:	Vaskuläre Regulationsmechanismen
GK 1123:	Cellular Mechanisms of Learning and Memory Formation as well as Memory Consolidation
GK:	Biophysical Investigations of Protein Interactions
GK:	International Max Planck Research School: The Life Course: Evolutionary and Ontogenetic Dynamics (LIFE)

Projects from the impulse and networking fund of the Helmholtz Association President

School laboratory
Virtual institute “HOBIT” (Helmholtz Open Bioinformatics Technology)
Virtual institute “Berlin Institute for Heart Research” (BIHR)
“Stroke Helix” / “Centred for Stroke Research Berlin”
Helmholtz Junior Group: Dr. Inés Ibañez-Tallon “Molecular Neurobiology”
Helmholtz University Junior Group: Dr. Frank Rosenbauer “Cancer stem cells and transcription factors”

Projekte aus dem Impuls- und Vernetzungsfonds des Präsidenten der Helmholtz-Gesellschaft

Schülerlabor
Virtuelles Institut „HOBIT“ (Helmholtz Open Bioinformatics Technology)
Virtuelles Institut „Berlin Institute for Heart Research“ (BIHR)
„Stroke Helix“ / „Centrum für Schlaganfall-Forschung Berlin“
Helmholtz-Juniorgroup: Dr. Inés Ibañez-Tallon „Molecular Neurobiology“
Helmholtz-Hochschul-Juniorgroup: Dr. Frank Rosenbauer „Cancer stem cells and transcription factors“

Technology Transfer

Since 2002, the MDC has worked together with the company Ascenion GmbH as a partner for the scientific utilization of the findings. An extensive infrastructure for technology transfer was created in order to accompany ideas and inventions professionally from their creation through to their realization and application.

Figures for technology transfer

Patent applications in 2004	6
Patent rights as of as of 31.12.2004	88
Licence agreements	17
Licence revenues	€ 666,000
R&D commissions (number)	4
R&D commissions (proceeds)	€ 234,000
R&D cooperations (number)	127
R&D cooperations (proceeds)	€ 804,000

At present, the MDC has holdings in three private companies.

Shareholdings in companies

Company	Registered office	Homepage
RZPD Deutsches Ressourcen-zentrum für Genomforschung GmbH	Heubnerweg 6 14059 Berlin	www.rzpd.de
BBB Management GmbH Campus Berlin-Buch (BBB)	Robert-Rössle-Straße 10 13125 Berlin	www.bbb-berlin.de
HELIOS Research Center GmbH (HRC)	Helios Klinikum Berlin Karower Str. 11 Haus 214 13125 Berlin	www.helios-kliniken.de

Technologietransfer

Seit 2002 arbeitet das MDC mit der Ascenion GmbH als Partner für die wirtschaftliche Verwertung der Ergebnisse zusammen. So wurde eine umfängliche Infrastruktur für den Technologietransfer geschaffen, um Ideen und Erfindungen von ihrer Entstehung bis zu ihrer Umsetzung und Anwendung professionell zu begleiten.

Kennzahlen zum Technologietransfer

Patenanmeldungen 2004	6
Schutzrechtsbestand per 31.12.2004	88
Lizenzverträge	17
Lizenzerträge	666 T€
FuE-Aufträge (Anzahl)	4
FuE-Aufträge (Erträge)	234 T€
FuE-Kooperationen (Anzahl)	127
FuE-Kooperationen (Erträge)	804 T€

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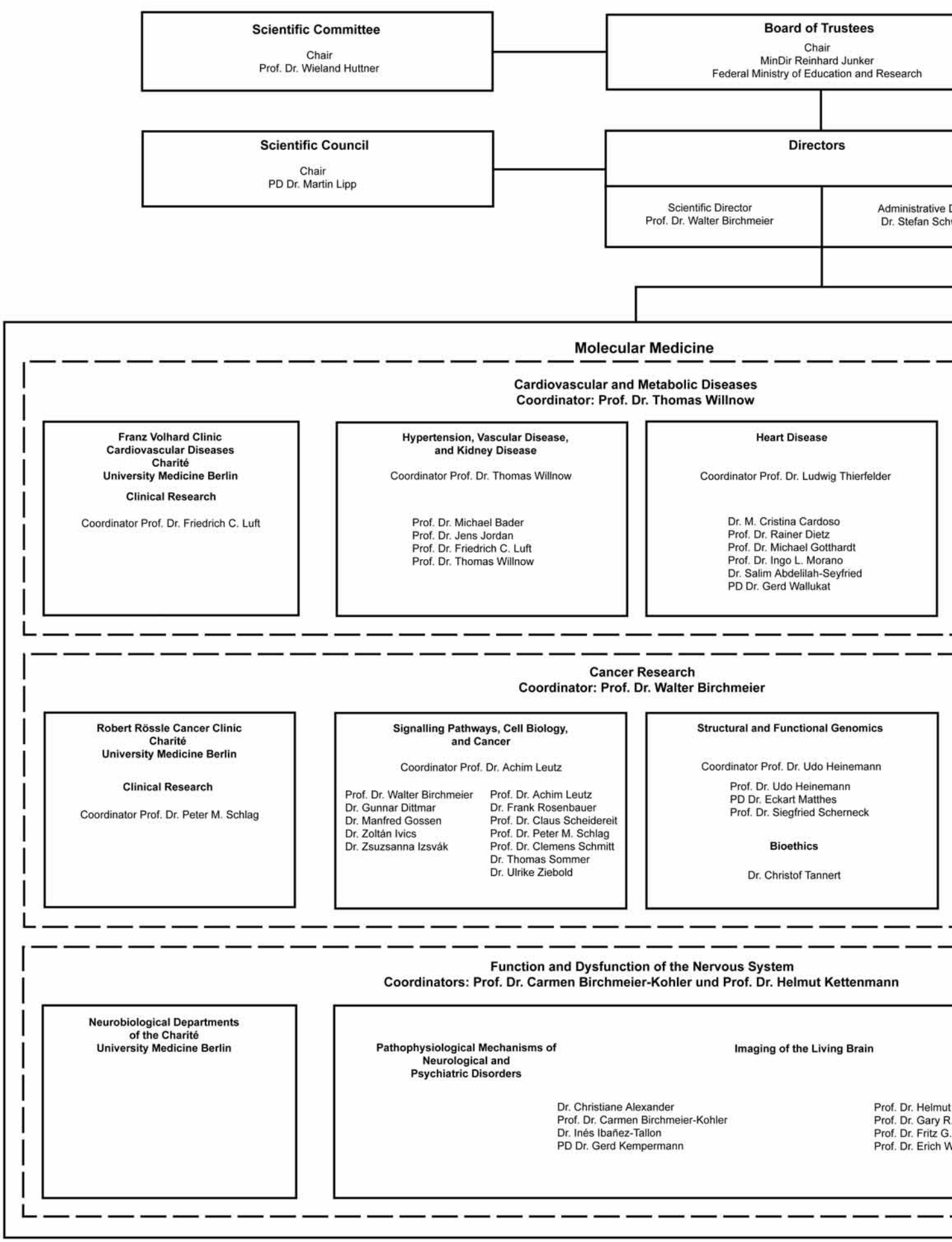
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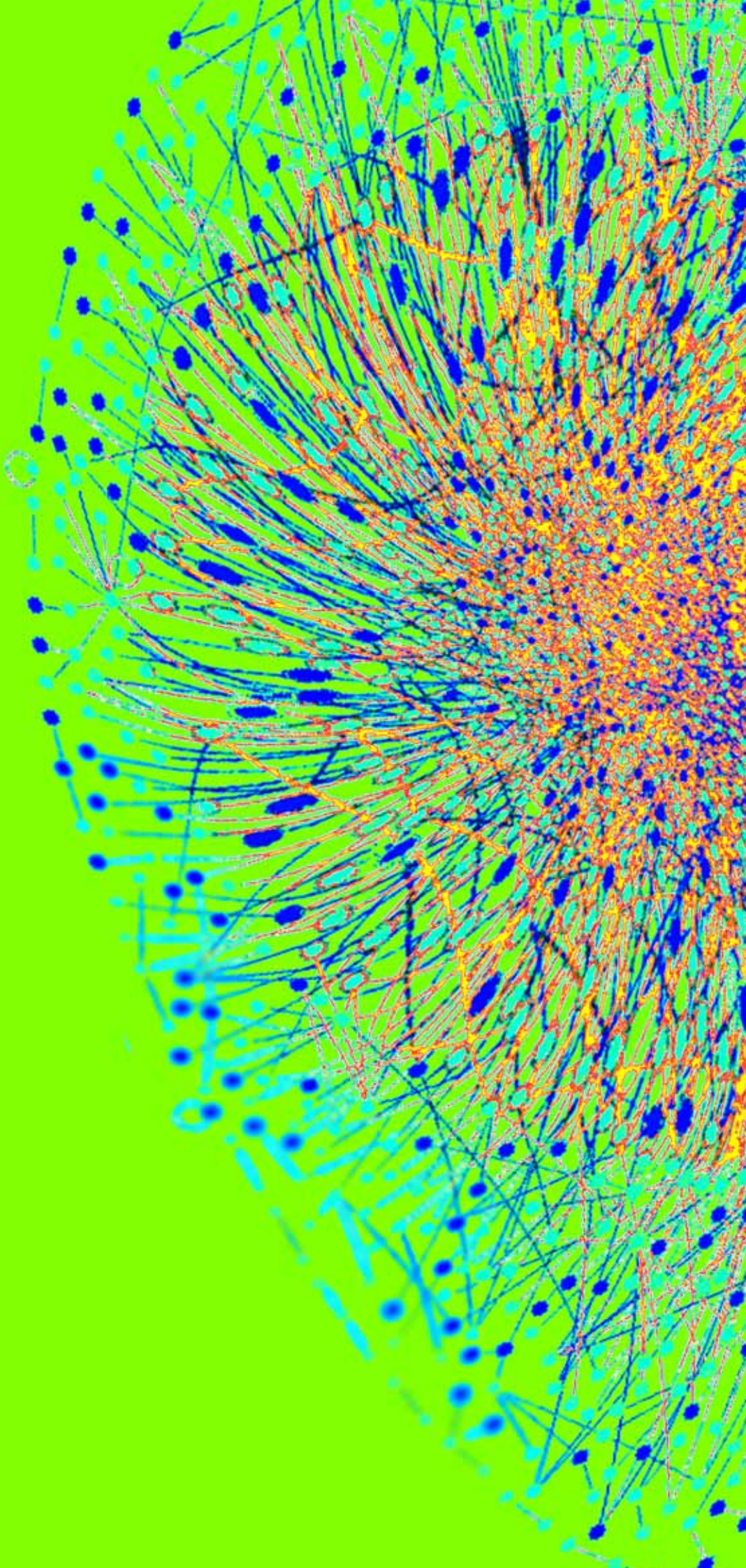

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