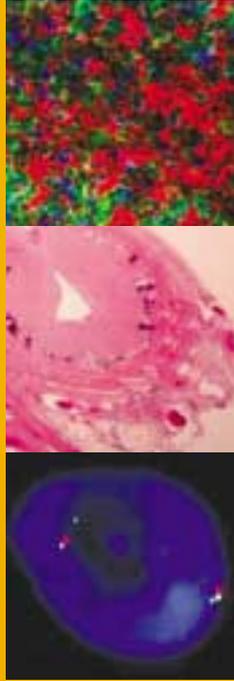


MDC
Berlin-Buch

Max Delbrück Center for Molecular Medicine

Research Report 2004



Research Report 2004

(covers the period 2002-2003)

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Foreword

We are pleased to present the 2004 Research Report of the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch, which covers the research period of the years 2002 and 2003. Founded in 1992, the MDC is a young research institute funded 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 (HGF)*). In addition to federal and state funds, the MDC augments its total research budget via third party financial resources. Indeed, with a sum of 11.2 million Euros, the MDC was ranked third of non-university research institutions in procuring grants from the German Research Association (*Deutsche Forschungsgemeinschaft (DFG)*). Furthermore, the Institute for Scientific Information (ISI/USA) National Citation Report placed the MDC among the top seven best molecular biological institutes in Germany.

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 collaboration between the MDC and the Buch Hospitals in the area of neurology is currently being expanded. Together with its partner institute, the Institute for Molecular Pharmacology (FMP) and the approximately 30 firms on the Buch Campus (in part new businesses founded by the MDC and the research clinics), the MDC has virtually the entire range of research and clinical tools for molecular medicine at its disposal. In addition to advances in genetics and cell biology, MDC scientists are able to analyze the structures of essential macromolecules and to develop interacting substances for potential therapeutic purposes. Such substances can then be tested in pre-clinical and clinical studies on the Buch Campus and, in some cases, engineered for use as clinical therapies.

Vorwort

Wir präsentieren Ihnen hiermit den wissenschaftlichen Bericht 2004 des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch, der die Forschungsperiode 2002 und 2003 umfasst. Das MDC ist ein junges Forschungsinstitut, gegründet 1992, das vom Bund und dem Land Berlin im Verhältnis 90 zu 10 getragen wird und zur Helmholtz-Gemeinschaft Deutscher Forschungszentren (HGF) gehört. Das MDC ist für seine Forschung stark auf die Einwerbung von Drittmitteln angewiesen, die einen großen Teil seiner effektiven Forschungsmittel ausmachen. Wie erfolgreich das MDC dabei ist, zeigt sich daran, dass es bei der Einwerbung von Fördermitteln bei der Deutschen Forschungsgemeinschaft (DFG) unter den außeruniversitären Forschungseinrichtungen mit 11,2 Millionen Euro Platz drei belegt. Weiterhin hat die Analyse des Institute for Scientific Information (ISI/USA) im National Citation Report gezeigt, dass das MDC inzwischen zu den besten sieben molekularbiologischen Instituten Deutschlands gehört.

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 dem Klinikum Buch im neurobiologischen Bereich werden im Moment ausgebaut. Zusammen mit dem Partner-Institut des MDC, dem Forschungsinstitut für Molekulare Pharmakologie (FMP), und den rund 30 Firmen auf dem Bucher Campus, die zum Teil Ausgründungen des MDC und der Forschungskliniken sind, verfügt das MDC somit über nahezu die ganze Bandbreite an Forschungswerkzeugen, um Krankheiten molekular zu charakterisieren. 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 können, um sie zum



„Junger Mann mit Schal“ (Portrait Max Delbrück) um 1935–40, Jeanne Mammen
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 „Young Man with Shawl“ (Portrait of Max Delbrück) about 1935-40, Jeanne Mammen
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This Research Report contains three parts which correspond to the three main research areas: cardiovascular diseases, cancer, and neuroscience. The research sections are intended for scientists and students, but also for potential new MDC collaborators. Organizational sections of the Research Report have been written in English and in German in order to make them accessible to the general public.

During the period of the Report, we were able to successfully carry out essential negotiations with important senior scientists at the MDC who had received offers from national and foreign universities and research institutes. Several MDC appointments of internationally recognized researchers are currently in process. In addition, during the 2002-2003 period, the three research areas of the MDC were evaluated by international experts through the Helmholtz Association (HGF) within the framework of the program-oriented research (PROF). The MDC received an excellent overall rating. Two strategic objectives are of future interest for the Institute. First, clinical research will be more intensively linked with basic research. Secondly, the collaborations between the MDC research programs and the university institutions will increase. To meet these objectives, the MDC is establishing the Experimental and Clinical Research Center (ECRC) on the Berlin-Buch Campus in cooperation with the Charité University Medical School Berlin.

We hope you enjoy reading the MDC Research Report 2004.

Detlev Ganten

Walter Birchmeier

Beispiel zu blockieren. Diese Substanzen können in vorklinischen und klinischen Studien getestet und verbessert werden und, so ist die Hoffnung, potentiell in der Diagnostik und Therapie von Patienten eingesetzt werden.

Sie sehen drei Teile im Wissenschaftlichen Bericht, die sich mit der Forschung in diesen drei Bereichen – Herz-Kreislauf-forschung, 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 der breiten Öffentlichkeit des MDC zugänglich zu machen. Ein zusätzlicher Teil beschäftigt sich mit neuen Entwicklungen in der Verwaltung und den technischen Abteilungen des MDC.

Wichtig war in der Berichtsperiode, dass wir erfolgreich wichtige Bleibeverhandlungen mit leitenden Wissenschaftlern des MDC führen konnten, die auswärtige Rufe erhalten hatten. Mehrere weitere Berufungen von auswärtigen, international anerkannten Forschern sind im Moment im Gange. Die drei Forschungsbereiche des MDC sind in der Berichtsperiode von der Helmholtz-Gemeinschaft im Rahmen der programmorientierten Forschung (PROF) international begutachtet worden, und sie haben exzellent abgeschnitten. Zukünftig werden zwei strategische Ziele von übergeordnetem Interesse für das Institut sein. Zum einen wird die Klinische Forschung stärker mit der grundlagenorientierten Forschung vernetzt werden. Zum zweiten werden die Forschungsprogramme die Zusammenarbeit mit den universitären Einrichtungen weiter ausbauen. Im Hinblick auf diese Ziele wird das MDC gemeinsam mit der Charité Universitätsmedizin Berlin ein neues Experimental and Clinical Research Center (ECRC) auf dem Campus Berlin-Buch errichten.

Wir wünschen Ihnen beim Studium dieses Forschungsberichtes viel Vergnügen.

Detlev Ganten

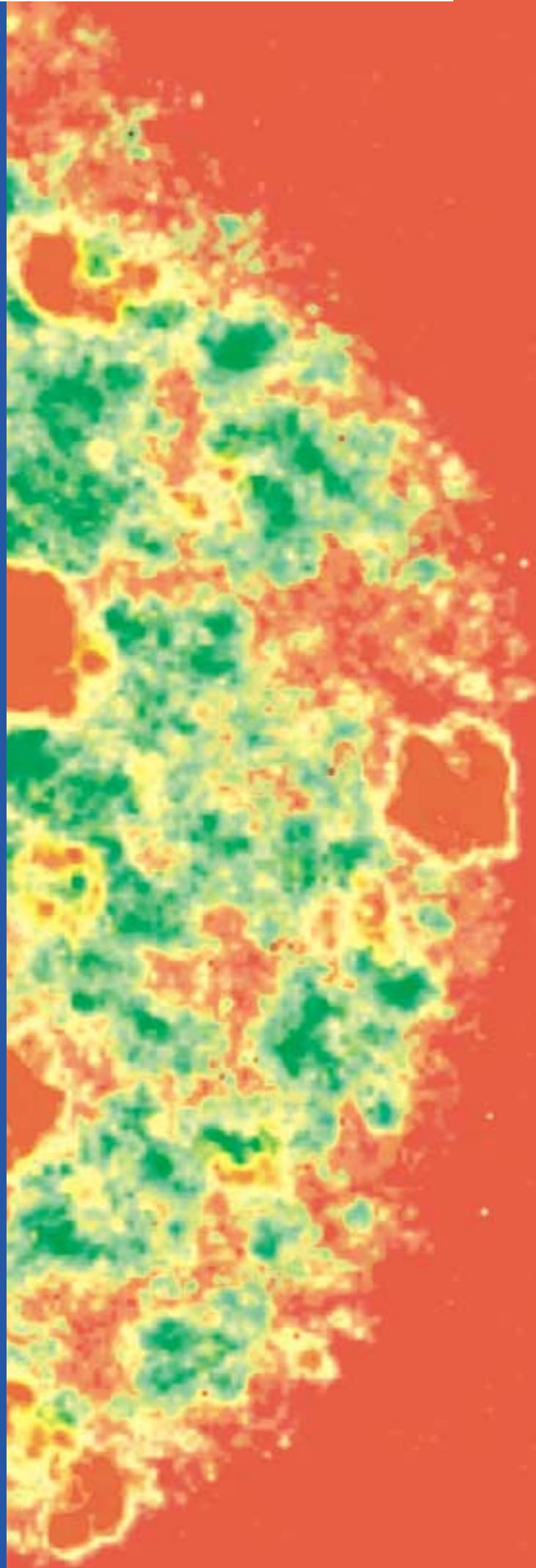
Walter Birchmeier

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

Thomas E. Willnow

Herz-Kreislauf- und Stoffwechselerkrankungen

Thomas E. Willnow

Introduction

Diseases of the cardiovascular and the metabolism are the major cause of morbidity and mortality in our society. Because such disorders particularly affect the elderly, the socio-economic 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 as well as those 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. Toward achieving these goals, we use functional genomics approaches to study disease processes in many systems that provide utilitarian models including fruit fly, zebrafish, frog, mouse, rat, and compare our findings to studies conducted in human (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). These research activities are coordinated in three topics that are of particular relevance to this program, namely:

- (1) Hypertension, Vascular and Kidney Diseases
- (2) Heart Diseases
- (3) Genetics, Genomics, Bioinformatics, and Metabolism.

Einführung

Erkrankungen des kardiovaskulären Systems und des Stoffwechsels sind die Hauptursache von Morbidität und Mortalität in unserer Gesellschaft. Auf Grund eines deutlichen Anstiegs der durchschnittlichen Lebenserwartung in unserer Bevölkerung und des erhöhten Risikos älterer Mensch 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 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 und in experimentellen Tiermodellen untersuchen und mit einander 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 engen Kooperation von Grundlagenwissenschaft am MDC und klinischer Forschung an der Franz-Volhard-Klinik für Herz-Kreislauf-erkrankungen (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, and Kidney Diseases

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, and kidney disease, new therapies may become possible. Major scientific achievements in this program topic in the last two years include the identification of novel signaling pathways in platelets involving serotonylation of small GTPases by the group of Michael Bader. In addition, Friedrich Luft and colleagues were able to fine map a gene locus on chromosome 12p responsible for autosomal-dominant hypertension, while Thomas Willnow and co-workers uncovered the molecular mechanism responsible for the renal uptake of aminoglycoside antibiotics paving the way for future strategies to prevent nephrotoxic side effects of these widely used therapeutics.

Heart Diseases

Coronary artery disease, myocardial infarction, heart failure, and cardiomyopathies are the main research areas in this topic. Primarily, we focus on the identification of disease genes that underlie monogenic traits in patients and in animal models as an approach to understand the molecular basis of heart disease. Major achievements in recent years include the identification of mutations in the muscle protein *titin* as a cause of hereditary cardiomyopathy in affected families (Ludwig Thierfelder and colleagues) and the pathophysiological characterization of this protein in a mouse model of *titin* deficiency (Michael Gotthardt). Salim Abdelilah-Seyfried's laboratory characterized the crucial role of atypical protein kinase C in determination of epithelial cell polarity and heart development, while Gerd Wallukat characterized autoantibodies against G-protein coupled receptors as a mechanism contributing to myocarditis, dilated cardiomyopathy, and hypertension in patients.

Genetics, Genomics, Bioinformatics, and Metabolism

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 projects. 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. Toward these goals, recent major achievements in this program have been the positional cloning of disease genes responsible for autosomal-recessive hypercholesterolemia (Friedrich Luft), familial hypertrophic cardiomyopathy (Peter Nürnberg and Karl-Josef Osterziel), as well as nephronophthisis, and related nephritic disorders (Peter Nürnberg).

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. Zu unseren bedeutendsten wissenschaftlichen Leistungen der vergangenen 2 Jahre zählen die Arbeiten der Gruppe um Michael Bader über die zentrale Rolle serotonin-vermittelter Prozesse bei der Blutplättchenaggregation und der Thrombose-Entstehung. Friedrich Luft und Kollegen gelang es, einen Genort auf Chromosom 12 zu kartieren, der eine erbliche, autosomal-dominante Form der Hypertonie beim Menschen bedingt. Der Verfasser dieses Beitrags (Thomas Willnow) und seine Mitarbeiter konnten den Mechanismus aufdecken, der für die Nephrotoxizität von Aminoglykosiden verantwortlich ist, eine lebensbedrohliche Nebenwirkung der Therapie mit diesen Antibiotika.

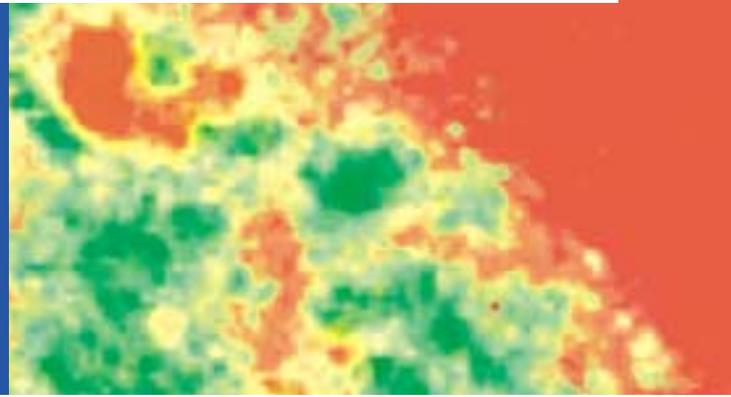
Herzkrankungen

Erkrankungen der Koronargefäße, Herzinfarkt, Herzversagen und Kardiomyopathien sind die Hauptforschungsfelder dieses Themas. Wir konzentrieren uns dabei vorrangig auf die Kartierung neuer Krankheitsgene, welche Ursache erblicher Herzkrankungen beim Menschen sind. Begleitend hierzu erstellen wir transgene Tiermodelle, welche dem Patienten vergleichbare genetische Veränderungen tragen und uns eine detaillierte Untersuchung pathologischer Vorgänge ermöglichen. Zu unseren wesentlichen Ergebnissen der letzten Jahre zählen der Nachweis von Mutationen im Muskelprotein Titin als Ursache einer erblichen Form der Kardiomyopathie beim Patienten (Ludwig Thierfelder und Kollegen) sowie die pathophysiologische Charakterisierung dieses Proteins in einem Mausmodell mit induziertem Titin-Gendefekt (Michael Gotthardt). Das Labor von Salim Abdelilah-Seyfried konnte die zentrale Rolle bearbeiten, welche die atypische Proteinkinase C bei der Regulation der Epithelzell-Polarität und der Herzentwicklung spielt. Gerd Wallukat hat Autoantikörper gegen G-Protein-gekoppelte Rezeptoren als Ursache dilatativer Kardiomyopathien identifiziert.

Genetik, Genomik, Bioinformatik und Metabolismus

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 und biostatistische Verfahren zu entwickeln, um die Flut genetischer Information zu analysieren und für funktio-

nelle Untersuchungen wie der Kartierung von Krankheitsgenen einzusetzen. Unsere Arbeiten der letzten Jahre haben sich im Wesentlichen auf die Anwendung dieser Technologien zur Hochdurchsatz-Analyse monogenetischer und komplexer genetischer Krankheiten fokussiert. Wichtigste Ergebnisse dieser Arbeiten waren die positionelle Klonierung der Gene für autosomal-rezessive Hypercholesterinämie (Friedrich Luft), für familiäre hypertrophe Kardiomyopathie (Peter Nürnberg und Karl-Josef Osterziel) sowie für Nephrophtise (Schrumpfniere) und verwandte nephritische Störungen (Peter Nürnberg).



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), massive increase in the concentration of circulating plasma lipoproteins results in hyperlipidemia and results 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 are designated members of the LDL receptor gene family (figure 1). 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 receptors of the LDL receptor gene family play in the (patho)physiology of the lipid metabolism. Towards this goal, we are using gene targeting approaches to generate mouse models with deficiencies in LDL receptor-related receptors and to analyze the consequences of the receptor gene defects *in vivo*. In recent studies, we have identified important new functions of lipoprotein receptors in bone metabolism, brain development and male fertility as well as the molecular mechanisms underlying human diseases in these areas.

Role of megalin in renal uptake of steroid hormones

We have focused on the functional characterization of megalin, a member of the LDL receptor gene family predominantly expressed in the neuroepithelium of the developing embryo and in proximal tubules of the adult kidney. Targeted disruption of the respective gene in the mouse results in developmental defects of the forebrain (holoprosencephaly) and in perinatal lethality of affected animals. Because we were particularly interested in elucidating the role of the receptor in the adult kidney, we used conditional gene targeting to generate mice with a kidney-specific megalin gene defect and to

circumvent the problem of perinatal lethality. Using mice with kidney-specific megalin deficiency, we identified megalin as a receptor for vitamin D binding protein (DBP), the plasma carrier for the steroid 25-(OH) vitamin D₃, and demonstrated that the receptor mediates the tubular retrieval of 25-(OH) vitamin D₃-DBP-complexes filtered through the glomerulus. This receptor-mediated uptake is required to prevent the loss of vitamin D₃ metabolites by glomerular filtration. Furthermore, it delivers 25-(OH) vitamin D₃ to tubular epithelial cells for conversion into 1, 25-(OH)₂ vitamin D₃, the active form of the vitamin and a potent regulator of the systemic calcium and bone metabolism. Urinary excretion of 25-(OH) vitamin D₃ in megalin-deficient mice results in vitamin D deficiency and in impaired bone calcification. Thus, megalin acts as an endocytic receptor for the uptake of lipophilic vitamin D and controls a central regulatory step in bone metabolism. Ongoing research is directed towards the elucidation of the role of megalin in forebrain development and in holoprosencephaly, a defect that may be caused by impaired embryonic cholesterol metabolism and that is among the most common developmental defects of the human embryo (1 in 250 pregnancies).

Role of megalin in aminoglycoside-induced nephrotoxicity

Besides clearance of endogenous ligands from the primary urine, megalin is also responsible for retrieval of foreign substances filtered through the glomerulus. This activity is particularly relevant for the renal uptake of therapeutic drugs that accumulate in the kidney causing nephrotoxicity. Such drugs include aminoglycosides, antibiotics commonly used to treat life-threatening gram negative bacterial infections. Aminoglycosides were known to accumulate in the renal proximal tubules causing nephrotoxicity and kidney failure. However, the pathway responsible for renal uptake of the antibiotic remained elusive. We have used mouse models with genetic megalin deficiency to explore the contribution of this receptor to renal aminoglycoside uptake *in vivo*. We demonstrated that the uptake of aminoglycosides into the kidney directly correlates with renal megalin activity and is completely eliminated in mice lacking the receptor. Thus, our studies provide unequivocal genetic evidence that megalin is the major pathway responsible for renal aminoglycoside accumulation and that the receptor represents a unique drug target to prevent aminoglycoside-induced nephrotoxicity. In collaboration with pharmaceutical partners, we are currently developing antagonists that block aminoglycoside binding to megalin and that may be used in the future to prevent aminoglycoside-induced kidney damage in patients.

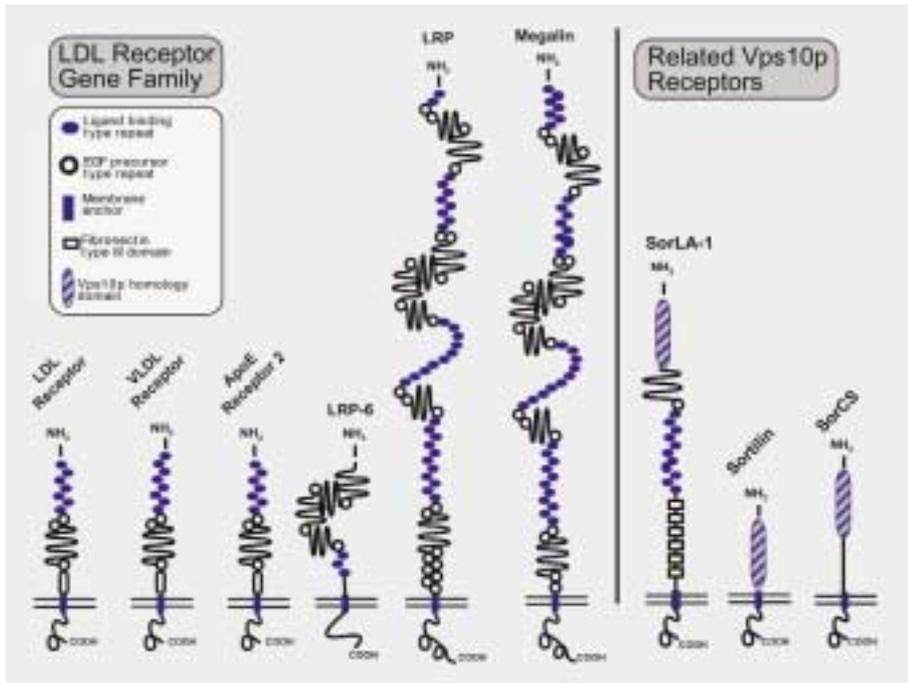


Figure 1) Structural organization of selected members of the LDL receptor gene family and related Vps10p receptors. Structural elements found in the various receptor species are depicted. These elements include ligand binding type (filled dots) and epidermal growth factor (EGF) precursor type repeats (open dots), transmembrane domains (filled square), fibronectin type III repeats (open square) and Vps10p homology domain (filled oval).

Role of the apolipoprotein receptor E-2 in sperm development

The apolipoprotein (apo)E receptor-2 (apoER2) is another member of the low-density lipoprotein receptor gene family and an important regulator of neuronal migration. Besides acting as a lipoprotein receptor, the protein also functions as a cellular receptor for the signaling factor Reelin and provides positional cues to neurons that migrate to their proper position in the developing brain. Loss of receptor activity in a knockout mouse model results in false layering of neurons in the central nervous system. Besides brain defects, apoER2-deficient mice also exhibit male infertility suggesting a as of yet unknown role of the receptor in male reproduction. We demonstrated that apoER2 is highly expressed in the initial segment of the epididymis where it affects the functional expression of phospholipid hydroperoxide glutathione peroxidase (PHGPx), an enzyme required for sperm maturation. Reduced PHGPx expression in apoER2 knockout mice results in the inability of the sperm to regulate the cell volume and in abnormal sperm morphology and sperm immotility (figure 2). Because insufficient expression of PHGPx is a major cause of infertility in men, these findings not only highlight an important new function for apoER2 that is unrelated to neuronal migration but also suggest a possible role for lipoprotein receptors in human infertility. Ongoing research activities are aimed at unraveling the molecular mechanisms causing male infertility in these conditions.

Functional characterization of cellular sorting receptors

Previously identified members of the LDL receptor gene family exhibit structural motifs found in the LDL receptor (figure 1). This observation suggests a role of these receptors in endocytosis of extracellular ligands, a hypothesis supported by our findings in receptor-deficient mouse models. 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 (figure 1). SorLA-1 in turn is highly homologous to a novel class of mammalian Vps10p-related receptor (sortilins, sorCS) (figure 1). Although, several of these receptors have been shown to bind ligands relevant for lipoprotein metabolism such as apolipoprotein E or lipoprotein lipase, the physiological role of these sorting receptors and their relevance in cellular and systemic lipid metabolism is unclear at present. Here, we have generated knockout mouse models lacking functional expression of the various members of this gene family. Using these models, we have uncovered the function of sortilin as a novel receptor for nerve growth factor in the peripheral nervous system. Nerve growth factor (NGF) regulates neuronal development through both survival and death signalling via two distinct receptors, TrkA and p75^{NTR}. Both NGF and its precursor proNGF are released by cells, but in contrast to NGF that induces cell survival, proNGF selectively promotes p75^{NTR}-mediated apoptosis. We demonstrated that Sortilin, a receptor expressed in proNGF-responsive tissues, is indispensable for the pro-apoptotic effect of proNGF. Sortilin binds to the pro-domain of proNGF whereas p75^{NTR} selectively binds to the mature NGF domain. Together, both receptors form a composite receptor binding

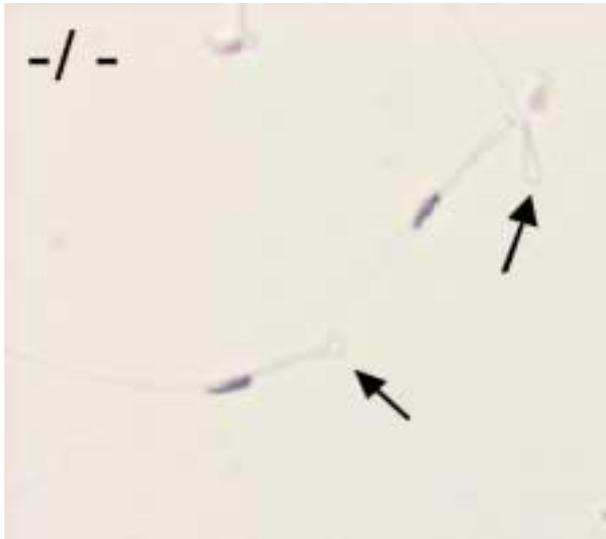


Figure 2) Abnormal sperm tail morphology in apolipoprotein receptor E-2 deficient mice. Sperm of apoEr-2 deficient mice (-/-) are characterized by abnormal bending of the sperm tail (arrows) causing sperm immotility and male infertility. Sperm of wild type mice (+/+) with normal straight tail morphology are shown for comparison.

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site on target cells responsible for mediating the pro-apoptotic effect of proNGF. Ongoing studies are aimed at identifying the role of the neurotrophin receptor Sortilin and related Vps10p receptors in the cardiovascular system.

Selected Publications

Nykjaer, A., Lee, R., Teng, T.T., Nielsen, M.S., Jansen, P., Madsen, P., Jacobsen, C., Kliemann, M., Willnow, T.E., Hempstead, B. and C.M. Petersen (2004). A novel neurotrophin receptor essential for proNGF induced neuronal cell death. *Nature* 427: 843-848.

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Molecular Biology and Genetics of Cardiovascular Diseases

Detlev Ganten

Mapping hypertension genes

The rat is one of the most important model systems for complex, polygenic diseases. We have demonstrated that multiple chromosomal loci in rat models contribute to blood pressure regulation and hypertension. Independent from elevated blood pressure, additional genetic factors contribute to end-organ damage and stroke in these animals. Ongoing research in our laboratory is directed towards the identification of the underlying predisposing genes and the subsequent identification of their molecular variants which cause different cardiovascular disease phenotypes. To localize disease genes within chromosomal regions linked to quantitative traits (e.g. blood pressure), we are establishing multiple congenic rat strains by introgressing disease alleles encompassing the quantitative trait locus (QTL) into a non-affected reference strain by successive backcrossing and molecular analysis. This strategy allows the observation of the effect and the genetic analysis of a single QTL. We are currently applying this strategy to a number of QTLs for blood pressure regulation, stroke, and kidney disease in the stroke-prone spontaneously hypertensive rat. A similar strategy is currently being adopted in collaboration with our Israeli partners to elucidate the genetic basis of salt-sensitive hypertension in the Sabra rat model. The combination of congenic experimentation with the development of subcongenic animals, with only a fraction of the initial congenic segment, will facilitate successive fine mapping within a QTL.

Analysis of the rat genome

As a partner in national and international rat genome research, our group has participated in the development of a large number of genomic resources for the rat to facilitate functional genomic studies in this species. These efforts are part of an international group of investigators led by the Baylor College of Medicine (Rat Sequencing Project Consortium) which have led to the sequencing and annotation of the rat genome.

Transgenic technology

In order to study the functional relevance of genes linked to hypertension and stroke, transgenic rats are produced with alterations in the expression of these genes. The power of this technology has been demonstrated in several transgenic rat models with modifications in the renin-angiotensin system. Rats expressing the mouse renin-2 gene (TGR(mREN2)27) have helped to reveal the physiological functions of local renin-angiotensin systems in tissues, in particular in the central nervous system.

It was shown that overactivation of the renin-angiotensin system in the brain markedly contributes to the pathogenesis of hypertension in this model. Furthermore, double transgenic rats carrying the human renin and angiotensinogen genes have been generated and turned out to be excellent models to study pregnancy-induced hypertension and hypertension-induced end-organ damage in kidney and heart. These rats are also used as suitable models for the development of renin inhibitors which represent a novel therapeutic approach to inhibit the renin-angiotensin system in hypertension and other cardiovascular diseases. In addition, numerous other transgenic rat models for the study of cardiovascular physiology and of the pathophysiology of cardiovascular and other diseases, such as Alzheimer's and Huntington's, have been generated and analyzed in collaboration with other groups.

Transgenic technology in the rat is developed further by the optimization of the methodology, the generation of transgenic animals with large genomic constructs, the use of additional strains of rats, and first steps toward the establishment of knockout technology for this species. It could be shown that superovulation of Sprague-Dawley rats can be achieved as efficiently by single injections of pregnant mare's serum gonadotropin as by minipump infusions of follicle stimulating hormone. Furthermore, the efficiency of transgenic rat production was shown to be independent of the transgene construct and overnight embryo culture did not diminish the success rate of the method. In addition to the most frequently used Sprague-Dawley strain, transgenic rats have been

Rat oocytes after somatic nuclear transfer



successfully generated from Wistar-Kyoto (WKY), Lewis, and spontaneously hypertensive rats (SHR-SP).

For the purpose of enabling knockout technology in the rat, the group has established embryonic stem (ES) cells from this species and has performed nuclear transfer experiments to allow the cloning of rats. Rat ES cells were developed from several strains including Sprague-Dawley and WKY but, despite expressing characteristic ES cell markers, these cells were not able to participate in the development of a rat embryo and, thus, could not be used to generate chimeras and, finally, knockout rats. However, the cells were shown to promote acceptance of transplanted organs when injected into host animals one week prior to transplantation, opening up a new therapeutic option using ES cells in transplantation medicine.

In order to enable the cloning of rats, an efficient culture system for rat preimplantation embryos was developed and enucleation and activation protocols for rat oocytes were optimized. First transfers of somatic cell nuclei into enucleated rat oocytes have produced embryos which, however, could only be cultured for a limited period of time. Nevertheless, the newly developed methods allowed the generation of tetraploid as well as parthenogenetic rat embryos as well as serve as a basis for the establishment of efficient nuclear transfer technology for the rat.

Selected Publications

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

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Medical Genomics

Norbert Hübner

Analysis of complex cardiovascular disorders in the rat

The rat is one of the most important model systems for complex, polygenic diseases. Since all epidemiologically important human diseases belong to this category, the potential for major advances through genetic investigation is substantial.

We have demonstrated that multiple chromosomal loci in rat models contribute to blood pressure regulation and hypertension. Independent from elevated blood pressure, additional genetic factors contribute to end-organ damage and stroke in these animals. Ongoing research in our laboratory is directed towards the identification of the underlying predisposing genes and the subsequent identification of their molecular variants, which cause cardiovascular disorders.

To localize disease genes within chromosomal regions linked to quantitative traits (e.g. blood pressure), we are establishing multiple congenic rat strains by introgressing disease alleles encompassing the quantitative trait locus (QTL) into a non-affected reference strain by successive backcrossing and molecular analysis. This strategy allows the observation of the effect and the genetic analysis of a single QTL. We are currently applying this strategy to a number of QTLs for blood pressure regulation, stroke, and kidney disease in the stroke-prone spontaneously hypertensive rat.

The combination of congenic experimentation with the development of subcongenic animals, with only a fraction of the initial congenic segment will enable successive fine mapping within a QTL. The mapping efforts of complex cardiovascular traits by congenic experimentation and positional cloning will be used in ongoing projects jointly with the establishment of gene expression signatures in target organs of congenic animals and their parental progenitors. High density microarrays are used for this approach. A combinatorial approach of positional cloning and expression profiling will provide a powerful tool to identify positional candidate genes within chromosomal regions for genetically determined cardiovascular diseases.

These data are being used to identify clusters of genes that co-segregate with well-documented cardiovascular and metabolic phenotypes within spontaneously hypertensive rats and to identify the underlying allelic variants. By determining the genetic networks and regulatory mechanisms underlying the observed patterns of gene expression, these data will provide new insights into the control mechanisms for hypertension, insulin resistance, and associated metabolic phenotypes that may be shared in common with similar disorders in humans.

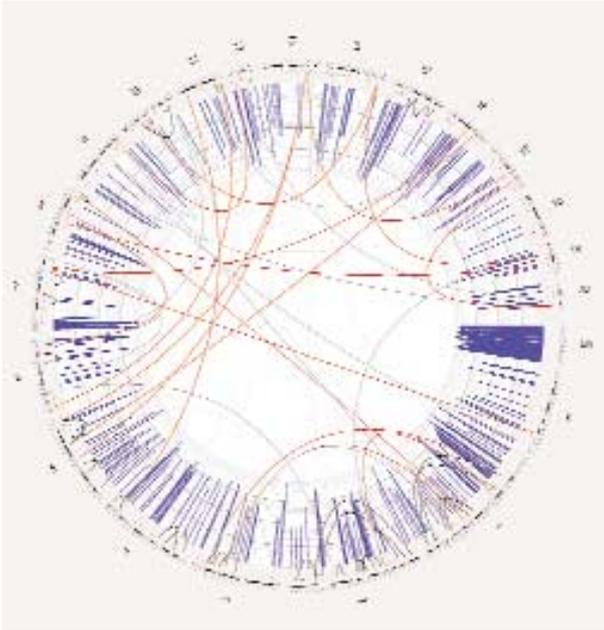
The identification of disease-relevant genes within QTLs by positional cloning will be greatly facilitated once the sequence of the entire rat genome is known. Moreover, functional studies often require access to clones in specific regions of interest. We have thus participated in the Rat Genome Sequencing Project Consortium, which resulted in the identification and annotation of the entire rat genome sequence. Additionally, we have built a physical map based on Yeast Artificial Chromosomes (YAC) and Bacterial Artificial Chromosomes (BAC). Combined this map comprises more than 200,000 BAC and YAC clones which are all anchored to the genomic sequence. These clones provide ready access to any genomic region for functional studies.

Selected Publications

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

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Anchoring of fingerprint and YAC map regions to sequence assembly.

YAC contigs are anchored to the assembly by way of hybridizations to BACs with sequence coordinates. The sequence coverage of anchoring BACs is typically less than the estimated size of the YAC contigs and therefore the contigs as drawn do not reflect actual size. Light grey lines link regions that are (a) are anchored by hybridization to a single BAC that is associated with <80% of the contig YACs or (b) are anchored by <20% of the contig BACs, leading to spurious contig segmentation on the assembly not likely to be due to actual inconsistencies between the YAC map and the sequence assembly. Red lines link the remaining region pairs, for which segmentation evidence is robust.

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Maolian Gong, Hongye Zhang, Herbert Schulz, Young-Ae Lee, Kai Sun, Sylvia Bähring, Friedrich C. Luft, Peter Nürnberg, Andre Reis, Klaus Rohde, Detlev Ganten, Rutai Hui, Norbert Hübner. (2003) Genome-wide linkage reveals a locus for human essential (primary) hypertension on chromosome 12p. *Human Molecular Genetics* 12:1273-1277.

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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 of these systems, 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 centrally involved in blood pressure regulation and, therefore, has been studied in detail employing transgenic techniques. A major focus of our research is local angiotensin-II generating systems in tissues such as brain, heart, and kidney. Transgenic rats expressing an antisense-RNA against angiotensinogen exclusively in astrocytes of the brain were produced and showed a decreased local concentration of this protein and lowered blood pressure and plasma vasopressin levels. Using these rats, we could show that central angiotensin modulates circadian blood pressure rhythms and the baroreceptor reflex. Furthermore, it is involved in the hypertensive and hypertrophic effects of circulating angiotensin. In a transgenic mouse model lacking angiotensinogen synthesis in heart and kidney, a role of local angiotensin-II generation in these organs could be demonstrated for the pathogenesis of hypertensive end-organ damage. Recently, new components of the RAS such as ACE2, the renin receptor, the mas protooncogene and angiotensin (1-7) have become a subject of research and transgenic animal models for the functional analysis of these molecules have been developed and characterized. Using knockout mice for the *mas* protooncogene, it could be shown that this protein is a functional receptor for angiotensin (1-7).

Kallikrein-kinin system

The kallikrein-kinin system (KKS) is an important hormone system for cardiovascular regulation mostly counteracting the effects of the RAS. As a model to study the functions of the

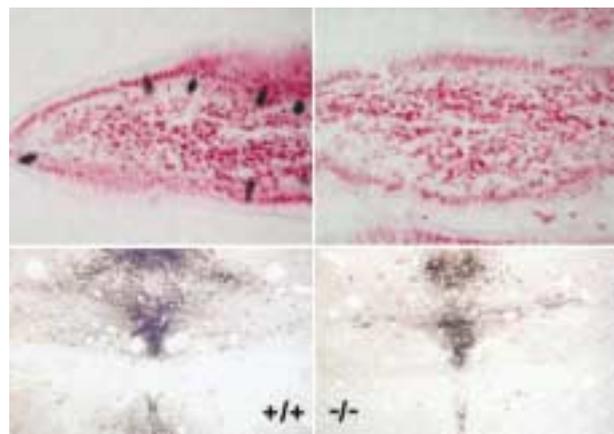
KKS in an intact animal, transgenic rats were generated expressing the human tissue kallikrein gene in all organs tested and excreting the protein in the urine. In these rats, blood pressure and its diurnal rhythmicity as measured by telemetry is significantly reduced compared to control rats. Moreover, kidneys and hearts of the animals are protected against ischemic and hypertrophic injury.

The functions of the kinin B1 receptor are enigmatic. Therefore, we generated mice lacking this subtype. The resulting animals exhibited analgesia, altered inflammatory reactions and reduced neovascularization, demonstrating an important role of the B1 receptor in pain transmission, inflammation, and angiogenesis. Recently, mice lacking both kinin receptors, B1 and B2, have been generated by deleting the B1 gene in embryonic stem (ES) cells derived from B2 knockout mice. These animals are totally unresponsive to kinins and show complete protection from septic-shock induced hypotension.

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. In order to functionally characterize this hormone, we deleted the gene encoding the rate limiting enzyme for its synthesis, tryptophan hydroxylase (TPH), from the mouse genome. The resulting mice were depleted from serotonin in the circulation but, surprisingly, showed normal serotonin levels in the brain. This led us to detect and characterize a second gene coding for TPH, TPH2, responsible for serotonin synthesis in the central nervous system. The TPH1 deficient mice exhibited defects in platelet function due to a blunted release of von Willebrand factor (vWF) at sites of vessel injury. Further analysis revealed that serotonylation of small GTPases is a novel and essential signalling pathway in the release of platelet α -granules.

Serotonin in intestine and brain of normal (left panels, +/+) and TPH1-deficient (right panels, -/-) mice. Serotonin is absent from chromaffin cells of the gut (upper panels) of TPH1-deficient mice but present in normal amounts in the Raphe nuclei of the brainstem (lower panels) due to the existence of a second tryptophan hydroxylase gene, TPH2, exclusively expressed in the brain.



Transgenic and stem cell technology

In collaboration with other groups, the expression of further proteins with relevance for cardiovascular and other diseases have been altered by transgenic and knockout technology in mice and rats, such as the liver fatty acid binding protein, smooth muscle myosin heavy chain, natriuretic peptide receptors, and huntingtin.

In order to allow gene-targeting experiments also in the rat, which is more suitable for the research on cardiovascular diseases than the mouse, we have established ES cells from this species and performed nuclear transfer experiments to allow the cloning of rats. However, the rat ES cells did not form chimeras when injected into blastocysts and are therefore not suitable for gene targeting experiments. Instead, they blunted transplant rejection when injected several days prior to an allogeneic heart transplantation.

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Genetics, Nephrology, Hypertension, and Vascular Injury

Friedrich C. Luft

Summary

The group is interested in molecular genetics of cardiovascular disease, pathogenesis of renal diseases, hypertension and vascular injury. Sylvia Bähring leads a team focusing on genetics of blood pressure regulation and lipid metabolism. Both Mendelian conditions and complex genetic diseases are being addressed. Ralph Kettritz is interested in small vessel disease, particularly anticytoplasmic antibody-(ANCA) induced vasculitis. He is currently focusing on neutrophil biology. Dominik N. Müller is relying on a transgenic rat model to study mechanisms of vascular injury. Volkmar Gross is pursuing systems biology in the mouse. Various genetically modified animals are at his disposal. Anette Fiebler is interested in vascular effects of aldosterone. Ralf Dechend is focusing on pre-eclampsia, a malignant form of hypertension during pregnancy.

Molecular genetics

Sylvia Bähring and associates showed that autosomal-dominant hypertension with brachydactyly is caused by a gene rearrangement on chromosome 12p (in press). The group relied on interphase FISH and were joined by Anita Rauch (University of Erlangen) in this endeavor. Rearrangements have been identified in several families with this condition. Mapping them precisely, cloning the breakpoints, and elucidating expressed sequence tags in the regions involved should enable the team to clone the responsible genes. Hussam Al-Kateb et al showed that autosomal-recessive hypercholesterolemia in a Syrian kindred is caused by a novel mutation in an LDL-receptor adaptor protein. He and his associates have expressed the mutation in HEK cells and proved that the resulting mRNA was defective. Hans Knoblauch and associates performed a single nucleotide polymorphism (SNP) screen in 13 lipid-relevant genes in a study of 250 German families (>100 000 genotypes in >1000 people). They were able to explain most of the genetic variance in low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and almost all of the genetic variance in

the informative LDL/HDL ratio. Their informative haplotypes are currently being tested in other populations. Monozygotic and dizygotic twins continue to be a focus of the group. Andreas Busjahn has founded a company (Health-TwiSt GmbH) focusing on the twin model in elucidating genetic variance. However, the close association with the Nephrology/Hypertension section is being maintained.

Vasculitis

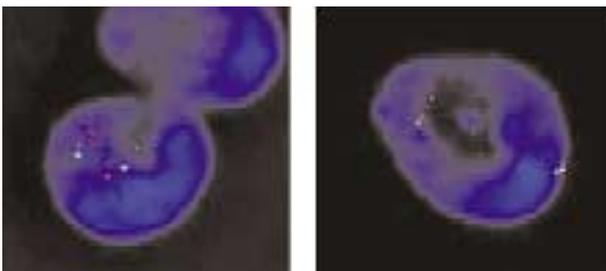
The group collaborated with the genetics group and relied on the twin model. They were able to show that ANCA expression on the neutrophil surface is highly influenced by genetic variance. Their findings raise the hope that genes regulating ANCA surface expression, and thereby susceptibility to the disease, can be isolated. Ralph Kettritz and Mira Choi were able to elucidate the functions of the nuclear factor Kappa-B (NF- κ B) in neutrophils by introducing a novel inhibitor (NEMO binding domain) into the cells. This feat was accomplished with help of the HIV protein TAT that is able to traverse cell membranes as a carrier of other molecules. Recently, the group showed that beta2-integrins provide co-stimulatory signals for matrix proteins that are able to activate NF- κ B in neutrophils. The signaling pathways involved are probably important in enabling neutrophils to tranverse across capillary barriers in inflammatory conditions.

Vascular injury

Dominik N. Müller and associates have convincingly demonstrated that angiotensin (Ang) II sets a series of events in motion that involve both innate and acquired immunity. The immune reactions signal inflammatory events that result in vascular injury. Dendritic cells are activated in this process, grow to maturity, and migrate as a consequence of Ang II-induced signaling. Blocking innate and acquired immunity, either pharmacologically or by depleting lymphocytes can ameliorate Ang II-induced vascular injury. In other studies, the group is focusing on eicosanoid participation in Ang II-induced vascular injury (cooperation with Wolf-Hagen Schunck). Aldosterone and the novel renin receptor have become recent focal points. Aldosterone signals via the mineralocorticoid receptor in cardiac and smooth muscle cells. The hormone augments Ang II-related effects. Reactive oxygen species and extracellular regulated kinase play a role in these responses.

Systems biology in mice

Volkmar Gross and Michael Obst have elucidated Ang II receptor 2 (AT₂) function with their work on AT₂ receptor knockout mice. The AT₂ receptor is important to shifts in pressure-natriuresis diuresis and also plays a role in the propensity to develop cardiac hypertrophy. The group is able to measure blood pressure and heart rate by telemetry and now have added continuous cardiac output measurements to their repertoire. They are currently focusing on soluble epoxide hydrolase knockout mice and mice with a disrupted RGS2 gene. The gene encodes a protein that regulates G-protein signaling.



The probes to BACs on chromosome 12p have been labeled with red, green, and yellow fluorescence. The figure shows that in these examples the markers are not in the same order on the two copies of chromosome 12p in the cells. This finding indicates that a rearrangement has taken place.

Preeclampsia

Ralf Dechend (Cardiology Section) is following a fascinating line of evidence involving activating AT1 receptor antibodies that appear before clinical signs of preeclampsia and that disappear after delivery. The group recently studied the effects of these autoantibodies on trophoblasts and vascular smooth muscle cells in terms of reactive oxygen species production. They convincingly showed that the antibodies activate the NADH oxidase in these cells and that the resulting reactive oxygen species stimulate the transcription factor NF- κ B. As a result, the cells produce various factors (e.g., tissue factor) that could contribute to the development of preeclampsia. Their work also raises potentially important therapeutic possibilities. An animal model is being developed.

Milestones

Arya M. Sharma has left the Nephrology/Hypertension section to assume a new position as director of a department for obesity studies at McMaster University, Ontario, Canada. His duties will be assumed by Jens Jordan (Helmholtz fellow), who has been appointed a Professor of Medicine (Clinical Pharmacology) at the Charité. Maik Gollasch (Helmholtz fellow) will assume new duties as Associate Professor of Physiology at the Louisiana State University, New Orleans, LA, USA. Both Arya Sharma and Maik Gollasch will maintain active associations with the Nephrology/Hypertension section in Berlin. Ralph Kettritz was recently appointed a Professor of Medicine (Nephrology) at the Charité.

Selected Publications

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Control of Smooth Muscle Cell Function

Maik Gollasch (Helmholtz Fellow)

The work in our lab focuses on the ionic mechanisms responsible for the onset and maintenance of intrinsic (myogenic) vascular tone of small arteries. A second area of research is directed towards identifying the role of the perivascular fat as a modulator of arterial tone, with specific emphasis on the resistance vasculature. A third area of research focuses on the dicarboxylate transporter encoded by the life-extending gene *Indy* (“I’m not dead yet”).

Calcium sparks and control of myogenic tone

Recent evidence indicates a role for subcellular calcium sparks as negative feedback regulators of arterial tone. Calcium sparks result from the concerted opening of a few ryanodine-sensitive calcium channels (RyR) in the sarcoplasmic reticulum. We use a combined approach, utilizing single cell isolation, ion channel recording techniques, and intracellular calcium as well as calcium spark measurements using conventional fluorescent imaging, and confocal laser scanning microscopy, diameter and membrane potential measurements in intact pressurized arteries, and expression of ion channels. Using gene knockout animals, we have been able to show that the $\beta 1$ -subunit (BK $\beta 1$) of the large-conductance calcium-activated potassium (BK) channel represents the molecular sensor of calcium sparks to reduce myogenic tone. Deletion of BK $\beta 1$ disrupts coupling between calcium sparks and BK channels, leading to increased arterial tone and systemic blood pressure in mice. We have shown that variants in the gene (KCNMB1) coding for the BK $\beta 1$ subunit are associated with baroreflex function in humans. Current work examines the role of canonical Transient Receptor Potential Channels (TRPC6, TRPC1) and L-type channels in release of calcium sparks and the role of the pore-forming BK α subunit in control of vascular tone. Malfunction of the calcium spark/BK channel pathway may be an important mechanism for the development of human hypertension.

Control of arterial tone by perivascular fat

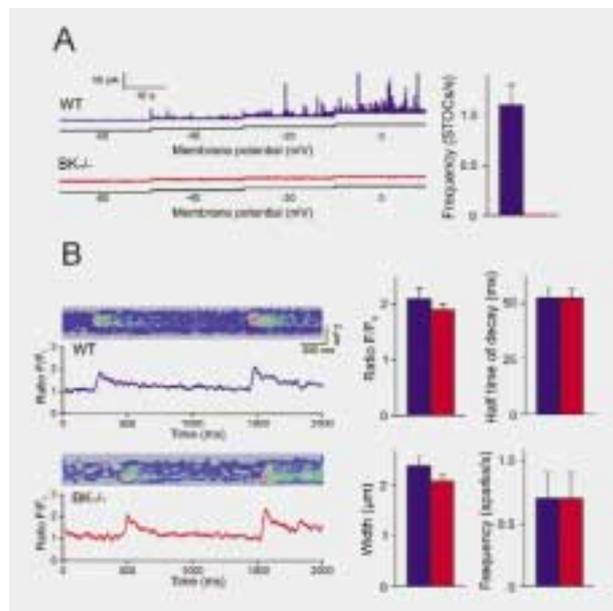
Virtually all blood vessels are surrounded by variable amounts of adipose tissue. Based on our results, we suggest that perivascular fat elaborates an adventitium-derived relaxing factor (ADRF). Release of ADRF is Ca^{2+} -dependent and is regulated by intracellular signaling pathways involving tyrosine kinase and protein kinase A. In small mesenteric arteries, perivascular adipose tissue induces vasorelaxation by activating smooth muscle delayed-rectifier K^+ channels. In collaboration with Dr. W.-H. Schunck’s group, we investigated the effects of P450-dependent epoxygenation of eicosapentaenoic acid on potassium channels. In collaboration with Drs. A. Otto and E.-C. Müller, we are currently examining the identity of “ADRF”. Perturbations of ADRF release and function could conceivably contribute to obesity-induced hypertension and to the development of arterial dysfunction in obesity and in other chronic vessel diseases.

Transport properties of *INDY*

Using two electrode voltage clamp and flux measurements in *Xenopus* oocytes, we have been 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. Current work examines transport mechanisms of *Indy* for tricarboxylates and *Indy* homologs in humans.

Calcium spark and spontaneous transient outward current (STOC) activity in freshly-isolated wt and BK α ^{-/-} cerebral arterial cells.

(A) STOCs were recorded at increasing membrane potentials, and STOC frequency at -20mV from 5-7 wt and BK α ^{-/-} cells. (B) Confocal line-scans of fluo-3-loaded wt and BK α ^{-/-} cerebral arterial cells, and time-course of corresponding Ca^{2+} sparks. Spark amplitudes were measured as local fractional fluorescence increases (F/F_0 ; F_0 is baseline). Spark duration was measured at half-maximal amplitude; $n=5-12$ randomly selected cells per genotype. All data are means \pm SEM; * $P < 0.05$; ** $P < 0.01$.



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Molecular Mechanisms of the Metabolic Syndrome

Jens Jordan (Helmholtz Fellow)

The main interest of the group is both mechanism- and patient-oriented research in the field of clinical autonomic disorders, arterial hypertension, obesity, and the metabolic syndrome. In the last few years, we established a clinical research center (CRC) to provide 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 (also called “metabolic syndrome”), orthostatic intolerance, autonomic failure, and neurogenic hypertension based on a better understanding of the pathophysiology of these clinical syndromes. We believe that studies on rare human diseases that are associated with low blood pressure may also give important insight into the mechanisms of essential and obesity associated hypertension. To elucidate the potential influence of candidate genes we conducted twin studies. We have a close collaboration with other groups at the MDC to confirm hypotheses that are generated in humans in already available or in newly created animal models.

Genetic influences on cardiovascular and metabolic regulation in health and disease

In earlier studies, we found a functional mutation in the norepinephrine transporter gene in patients with familial orthostatic intolerance. We further elucidated the role of the norepinephrine transporter in cardiovascular and metabolic regulation in a series of human pharmacological experiments. We found that norepinephrine transporter inhibition causes a selective impairment in sympathetic vasomotor regulation, which is suggestive of a central nervous sympatholytic effect. The baroreflex impairment resulted in hypersensitivity to vasoactive drugs. Furthermore, norepinephrine transporter inhibition elicited metabolic changes, both systemically and at the adipose tissue level. In particular, we found impaired lipid utilization. Our observations attest to the importance of the norepinephrine transporter in metabolic and cardiovascular regulation. They may have important implications for the

clinical use of norepinephrine transport inhibitors. We participated in studies on the pathophysiology of monogenic hypertension and brachydactyly. Sylvia Bähring's group showed that the syndrome is caused by a complex genetic rearrangement. We used systemic and intra-arterial infusions of various pharmacological agents to confirm or exclude candidate genes, such as PDE3A. Venous dysfunction contributes to many common cardiovascular diseases, such as varicosis, thrombosis, orthostatic intolerance, and, perhaps, arterial hypertension. In our twin studies, we found evidence for a strong genetic influence on venous function. Currently, we are analyzing the heritability of metabolic parameters, such as free and bound leptin and adiponectin concentrations.

Adipose tissue and the pathogenesis of obesity-associated cardiovascular disease

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 and leptin. Leptin circulates in both a receptor-bound and in a free form. Bound and free leptin appear to have different biological functions. We found a strong correlation between sympathetic nerve traffic and bound leptin concentrations in normal weight men. In contrast, sympathetic activity was not related to free leptin concentrations. The physiological role of angiotensin II in adipose tissue is poorly understood. We applied angiotensin II to the interstitial space in both adipose tissue and skeletal muscle using the microdialysis technique. Remarkably, we did not see a major change in tissue blood flow. Yet, angiotensin II changed lipid and carbohydrate metabolism in a tissue-specific fashion. The metabolic effect of angiotensin II might contribute to insulin resistance and explain, in part, the beneficial effect of angiotensin II inhibition on carbohydrate metabolism. We are continuing these studies in obese patients with and without arterial hypertension. We combine pharmacological methods, functional metabolic studies, and adipose tissue gene expression analysis. These studies are conducted in close collaboration with the adipocytes biology laboratory (Dr Engeli and colleagues).

Selected Publications

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Functional Characterization of Newly Identified Human Importin α Proteins

Matthias Köhler (Helmholtz Fellow)

Our group is working on nucleocytoplasmic protein transport. We have identified, in collaboration with Enno Hartmann and Dirk Görlich, several novel human isoforms of the importin α protein family, which mediate nucleocytoplasmic protein import in complex with importin β . Using in-vitro import assays, we have elucidated the import pathways of different import substrates and could demonstrate that the α importins differ in their substrate specificity in-vitro. Our group is further working on the identification of the in-vivo relevance of the different α importins. Our doctoral fellows, Maite Hartwig and Sebastian Thiel, showed that the α importins are differentially expressed in various models of cellular proliferation and differentiation. In collaboration with the group of Ilkka Julkunen from Helsinki, Finland, our doctoral fellow, Jacqueline Franke, investigated the import mechanisms of STAT proteins. Together, we demonstrated that activated STATs'1 and 2 strongly bind to importin $\alpha 5$ but not to other α importins. Furthermore, we identified the nuclear localization binding sites for STAT1, STAT2 and influenza A virus nucleoprotein on importin $\alpha 5$. A recent collaboration with the group of Mike Fainzilber, Weizmann Institute of Science, Rehovot, Israel, investigated the role of the importins in retrograde transport of signaling molecules in injured neurons. We showed that inhibition of the importin α/β transport complex

leads to a strong decrease in regenerative sprouting of neurites from lesioned neurons. The data obtained support a model whereby importin β , which is newly synthesized in injured neurons, mediates in complex with importin α and the motor protein dynein retrograde transport of signaling proteins from the injury site to the axoplasm, regulating repair mechanisms of lesioned nerves. Beate Friedrich, Christina Quensel, and Tanja Schmidt, doctoral and postdoctoral fellows in our group, are working on the identification of specific functions of the α importins in living cells and organisms.

Selected Publications

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Structure of the Group

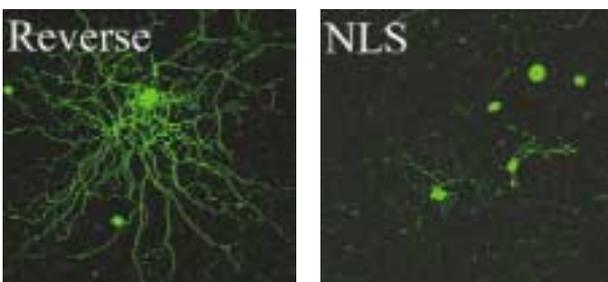
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Impairment of in-vitro regenerative neuronal outgrowth by inhibition of importin α/β mediated retrograde transport of signaling proteins via excessive addition of NLS-peptides.



Obesity and Hypertension

Arya M. Sharma

Summary

Obesity is the most rapidly growing public health problem worldwide. The condition commonly leads to cardiovascular disease and features the well-known risk factors of hypertension, diabetes mellitus (metabolic syndrome), and lipid disturbances. In many developed countries, thirty to forty percent of the population is overweight or obese. In the United States, this number is currently about 50% and is growing yearly. The condition is strongly influenced by genetic variance. Adipose tissue has revealed itself as amazingly diversified in terms of producing cytokines, chemokines, and hormones, as well as being a target for these molecules. Our group has directed its research efforts at elucidating the relationship between obesity and cardiovascular disease.

Adventitia-derived relaxing factor

Virtually all blood vessels are surrounded by adventitial fat. Adipocytes produce a host of vasoactive substances that may influence vascular contraction. We tested whether perivascular adipose tissue modulates contraction of aortic ring preparations. We studied aortic rings surrounded by periadventitial adipose tissue from adult Sprague-Dawley rats. At maximum concentrations of angiotensin II, serotonin, and phenylephrine, the contractile response of intact rings was, respectively, 95%, 80%, and 30% lower than that of vessels without periadventitial fat. The anticontractile effect of periadventitial fat was reduced by inhibition of ATP-dependent K⁺ channels with glibenclamide and by the tyrosine kinase inhibitor genistein. Blocking NOS, cyclo-oxygenase, cytochrome P450, or adenosine receptors did not restore the vascular response in intact vessels. The anticontractile effect of perivascular fat was present in Zucker fa/fa rats, suggesting that leptin receptors were not responsible. Transferring the bath solution from intact vessels, isolated periadventitial tissue, and cultured rat adipocytes to precontracted vessels lacking periadventitial fat resulted in a rapid relaxation. We suggest that perivascular adventitial adipose tissue releases a transferable adventitium-

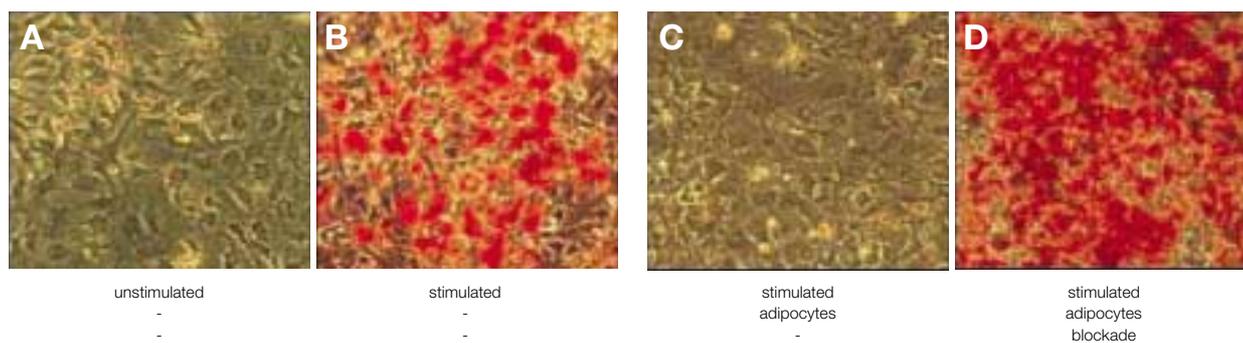
derived relaxing factor that acts by tyrosine kinase-dependent activation of K⁺ channels in vascular smooth muscle cells. Maik Gollasch and his group are following up on these studies (see report by M. Gollasch).

Adipocytes, preadipocyte differentiation and angiotensin (Ang II)

Recent studies suggest that Ang II plays a role in the adipogenesis of murine preadipocytes. We isolated preadipocytes from human adipose tissue and stimulated them to differentiate. Quantitation of gene expression during adipogenesis was performed for renin-angiotensin system genes. We added angiotensinogen, Ang II, or angiotensin receptor antagonists to the differentiation medium. We also examined the influence of adipocytes on adipogenesis by co-culture experiments. Stimulation of the Ang II type 1 receptor by Ang II reduced adipose conversion, whereas blockade of this receptor markedly enhanced adipogenesis. Adipocytes were able to inhibit preadipocyte differentiation in the co-culture. The effect was abolished by blockade of the Ang II type 1 receptor. This finding suggested a functional role of the renin-angiotensin system in the differentiation of human adipose tissue. Because angiotensinogen secretion and Ang II generation are characteristic features of adipogenesis, we postulated a paracrine negative-feedback loop that inhibits further recruitment of preadipocytes by maturing adipocytes. Our hypothesis is supported by *in vivo* human studies.

Adiponectin and inflammation

Low plasma levels of the adipocyte-produced anti-inflammatory factor adiponectin characterize obesity and insulin resistance. To elucidate the relationship among plasma levels of adiponectin, adiponectin gene expression in adipose tissue, and markers of inflammation, we obtained blood samples, anthropometric measures, and subcutaneous adipose tissue samples from 65 postmenopausal healthy women. Adiponectin plasma levels and adipose-tissue gene expression were significantly lower in obese subjects and inversely correlated with obesity-associated variables, including high-sensitive C-reactive protein (hs-CRP) and interleukin-6 (IL-6). Despite adjustment for obesity-associated variables, plasma levels of adiponectin were significantly correlated with adiponectin gene expression. Furthermore, the inverse correlation between plasma levels of hs-CRP and plasma adiponectin remained significant despite correction for obesity-associated variables, whereas the inverse correlation between adiponectin plasma levels or adiponectin gene expression in adipose tissue with plasma IL-6 were largely dependent on the clustering of obesity-associated variables. In conclusion, our data suggest a transcriptional mechanism leading to decreased adiponectin plasma levels in obese women and demonstrate that low levels of adiponectin are associated with higher levels of hs-CRP and IL-6, two inflammatory mediators and markers of increased cardiovascular risk. Stefan Engeli is currently responsible for the adipocyte group. They are investigating the regulation of adiponectin and other adipocyte products in obese persons before and after weight loss.



Undifferentiated preadipocytes (A) do not store lipids, but when stimulated (B) they differentiate and synthesize triglycerides. When exposed to Ang II (C), preadipocytes do not differentiate. Blocking the actions of Ang II by an Ang II receptor blocker (D) allows adipocyte differentiation to occur.

Pharmacological treatment of obesity

Sibutramine, a serotonin and norepinephrine transporter blocker, is widely used as an adjunctive obesity treatment. However, its effect on cardiovascular regulation is imperfectly defined. We collaborated with Jens Jordan's group to elucidate sibutramine's actions. In 11 healthy subjects, we compared the effect of sibutramine or matching placebo on cardiovascular responses to autonomic reflex tests and to a graded head-up tilt test. In addition, we tested sibutramine in combination with metoprolol. Testing was conducted in a double blind and crossover fashion. Sibutramine increased upright blood pressure and upright heart rate. This effect was abolished with metoprolol. The blood pressure response to cold pressor and handgrip testing was attenuated with sibutramine compared with placebo. Sibutramine also decreased low-frequency oscillations of blood pressure and plasma norepinephrine concentrations in the supine position. Our study showed that the cardiovascular effect of the antiobesity drug sibutramine results from a complex interaction of peripheral and central nervous system effects. The inhibitory clonidine-like action of sibutramine on the central nervous system attenuates the peripheral stimulatory effect.

Milestones

Arya Sharma has assumed new duties as chairman of a department for Obesity Research at McMaster University, Hamilton, Ontario, Canada. However, obesity related hypertension continues to be a research focus of the Nephrology/Hypertension section of the Franz Volhard Clinic. Jens Jordan will assume responsibility for the group. A close relationship between Arya Sharma and the Nephrology/Hypertension section is being maintained.

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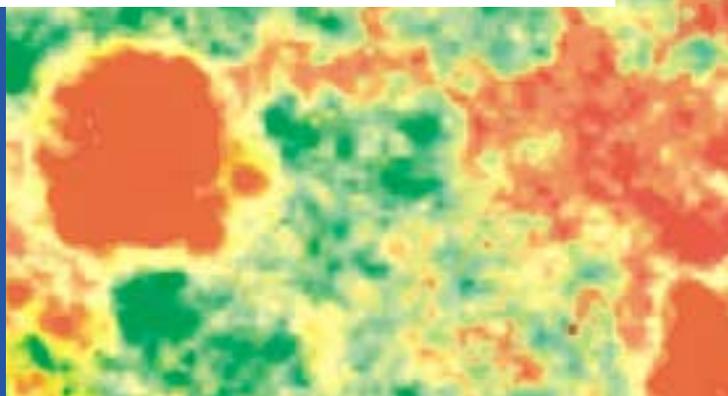
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Cardiovascular Molecular Genetics

Ludwig Thierfelder

Familial forms of heart failure

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. The most common CMP, dilated cardiomyopathy (DCM), is caused by autosomal dominant mutations in 20-30% of cases. A number of DCM disease genes have been identified that fall into different functional classes. This suggests that different disease pathways can culminate in the development of congestive heart failure due to progressive myocardial failure.

Little is known about the prevalence of mutations in individual DCM disease genes. In two DCM families, positional cloning efforts in our laboratory have recently led to the identification of mutations in titin (*TTN*) causing one form of non-syndromic DCM. Titin is the largest known molecule in mammals (3-3.7MDa) and encoded by a cDNA of up to 100kb. A truncation mutation of A-band titin and a missense mutation in I-band titin cause a similar phenotype in two unrelated families. Interestingly, although both mutations are expressed in cardiac and skeletal muscle, only cardiac muscle is clinically affected. Titin molecules extend from sarcomeric Z-discs to M-lines, provide an extensible scaffold for the contractile machinery and are critical for myofibrillar elasticity and integrity.

In a large DCM kindred, a segregating 2bp insertion mutation in titin exon 326 causes a frame shift, thereby truncating A-band titin. The truncated \approx 2MDa protein is expressed in skeletal muscle but Western blot studies with epitope-specific anti-titin antibodies suggest it undergoes 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 appears 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 another

large family with DCM also linked to CMD1G at chromosome 2q31, a titin 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. The identification of mechanisms of titin mutations should provide further insights into the pathogenesis of familial forms of congestive heart failure and myofibrillar titin turnover.

Isolated non-compaction of the left ventricle in the adult

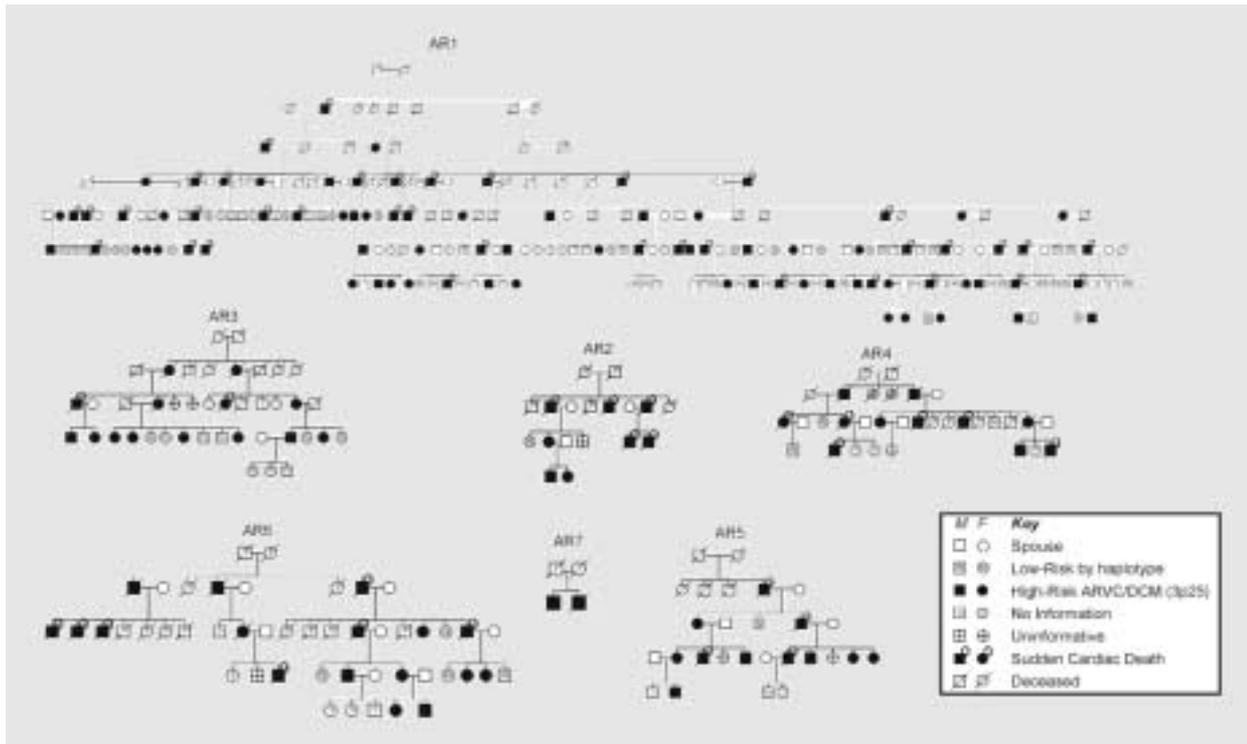
Isolated non-compaction of the left ventricle (INVC) is a rare disorder characterized by wide intertrabecular spaces due to an arrest of endomyocardial morphogenesis. It is well-known that infantile INVC is an X-chromosomal disease and caused by mutations in G4.5, a gene with a yet unknown function. We study 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 an autosomal locus was identified in a genomewide linkage analysis. Analyses of the putative disease gene are underway.

Malignant ventricular arrhythmias in a large Canadian founder population

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a difficult-to-diagnose cardiac condition associated with sudden death and heart failure. We have identified an autosomal dominant founder mutation on chromosome 3p25 in a large Canadian cardiomyopathy population of circa 400 individuals at a 50% risk of inheriting the condition. ARVC in these individuals is associated with a distinct electrocardiographic pattern and a life expectancy of males of less than 40 years. Mutational analyses of approximately 15 genes located in a 3Mbp region on chromosome 3p25 are in progress.

Molecular genetics of pseudoxanthoma elasticum (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



Seven pedigrees from a large Newfoundland population suffering from an autosomal dominant cardiomyopathy causing early sudden cardiac death, especially in men. All affected individuals share a founder mutation on chromosome 3p25.

have mapped the PXE locus to a 500 kb interval on chromosome 16p13.1 and have shown that mutations in a transmembrane transporter protein, ABC-C6 (also known as MRP-6), cause PXE.

Selected Publications

Gerull B, Gramlich M, Atherton J, McNabb M, Trombitás K, Sasse-Klaassen S, Seidman JG, Seidman CE, Granzier H, Labeit S, Frenneaux M, Thierfelder L. (2003). Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nat Genet* 30(2):201-4.

Sasse-Klaassen S, Gerull B, Oechslin E, Jenni R, Thierfelder L. (2003). Isolated noncompaction of the left ventricular myocardium in the adult is an autosomal dominant disorder in the majority of patients. *Am J Med Genet.* Jun 1;119A(2):162-7.

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Genetic Disorders of the Cardiovascular System

Brenda Gerull (Helmholtz Fellow)

Titin's role in heritable cardiac and skeletal muscle disorders

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.

Several inherited human muscle disorders have been mapped to the *TTN* locus on chromosome 2q31. One form of dilated cardiomyopathy (CMD1G), tibial muscular dystrophy (TMD), and proximal myopathy with respiratory failure (all autosomal dominant disorders) have been mapped to the *TTN* locus on chromosome 2q31. For CMD1G and TMD, *TTN* mutations have been identified by us and others. One of the CMD1G mutations is a complex mutation causing a frame shift in A-band titin (thereby introducing a premature stop codon) and, depending on muscle tissue, different posttranscriptional modifications of the truncated protein. In skeletal muscle, the truncated titin (expected size approximately 2MDa) is proteolytically digested to a 1,14MDa protein containing Z-disc and (partial) I-band titin. The truncated titin, however, is not present in a cardiac biopsy sample from an affected family member suggesting differences in posttranscriptional modifications. The question of whether the human cardiac phenotype in CMD1G is due to haploinsufficiency (as suggested by the Western blot results) and why no clinical phenotype of skeletal muscle is observed remain uncertain.

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

The antiphospholipid antibody syndrome (APS) is a complex, usually acquired 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 (referred to as 'Kindred A') with multiple members clinically affected by one or several 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 (presence of antiphospholipid antibodies; stillbirth, etc.) have not been mapped yet. We are currently screening for the chromosome 10p12 gene carrying the mutation responsible for thrombozytopenia in Kindred A.

Selected Publications

Sasse-Klaassen S, Gerull B, Oechslin E, Jenni R, Thierfelder L. (2003) Isolated noncompaction of the left ventricular myocardium in the adult is an autosomal dominant disorder in the majority of patients. *Am J Med Genet.* 1;119A(2):162-7.

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Myocardial Regeneration

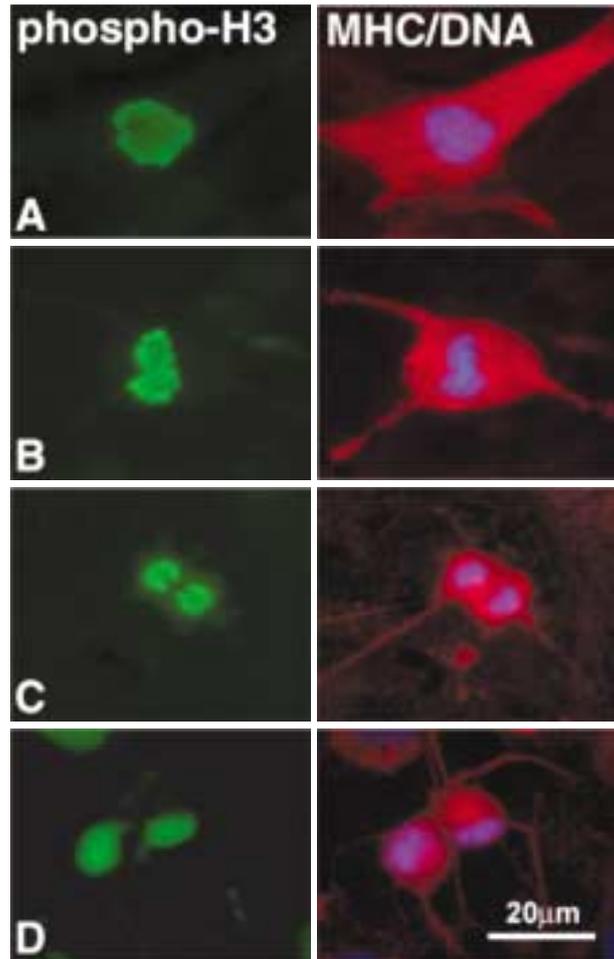
Rainer Dietz

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. So far, conventional therapy given to patients with heart failure aims at the reduction of the hemodynamic load in order to alleviate cardiac work. The establishment of a molecular approach to reinstall cardiomyocyte cell division would revolutionize standard treatment regimens for heart failure patients.

In general, there are two possibilities to prevent loss of cardiac contractile tissue after myocardial damage: 1) prevention of cell death, and 2) reinduction of cell cycle activity in surrounding healthy cardiomyocytes. Using mainly cell culture models of primary cardiomyocytes our group tries to identify pro- as well as anti-apoptotic signalling pathways in different forms of cardiomyocyte apoptosis. As a consequence of both acute and chronic myocardial damage, in most instances, detrimental cardiomyocyte hypertrophy develops. However, the intracellular pathways responsible for this myocardial maladaptive growth still remain enigmatic. Therefore, the intercalation between pro- and anti-apoptotic pathways on one side and classical growth cascades including cell cycle pathways on the other is also in the focus of our research interest.

Cardiomyocyte apoptosis requires cell cycle activation and downregulation of cell cycle inhibitors

We have found that both cyclin-dependent kinase inhibitors p21CIP1 and p27KIP1 need to be downregulated in order to trigger apoptosis in cardiomyocytes. Also cardiomyocyte apoptosis is characterized by activation of the pRb/E2F-dependent pathway. Moreover, yeast two hybrid screening of a human heart library revealed that the transcription factor E2F1, which previously has been shown by us and other groups to act in a pro-apoptotic fashion in primary cardiomyocytes, interacts with the ETS-related transcription factor GABP γ 1. This interaction links growth and cell death related



Ectopic E2F2 induces cell cycle reentry and mitosis in cardiomyocytes. Cardiomyocytes were infected with adenovirus wild-type E2F2 (100 pfu/cell) and continued to be cultivated for 48 hours. For the detection of M-phase specific phosphorylation of Histone H3, fixed cells were stained with rabbit polyclonal antibody to phospho-Histone H3, which recognizes serine 10 phosphorylation of Histone H3 (green). Cardiomyocytes were identified by costaining for the expression of MHC (MF20, red), and genomic DNA (Hoechst 33258, blue). Description of mitotic figures identified in cardiomyocytes: A, prophase; B, metaphase; C, early telophase; D, late telophase.

pathways in cardiomyocytes. More importantly, this is the first observation of a pRb-independent mechanism regulating E2F1-dependent transcription and apoptosis.

Phosphorylation by protein kinase CK2: A signalling switch for the caspase-inhibiting protein ARC

ARC, a recently discovered anti-apoptotic factor, the expression of which appears to be restricted to cardiac and skeletal muscle tissue, was found by our group to be a substrate of the casein kinase II (CK2). Constitutive phosphorylation of ARC by CK2 is required for ARC to act in an anti-apoptotic fashion.

p21CIP1 controls proliferating cell nuclear antigen protein level in adult cardiomyocytes

While trying to understand how cell death of cardiomyocytes is triggered, much effort of our group is devoted to decipher-

ing the regulation of cardiomyocyte cell cycle withdrawal. Employing different models, we have found that p21CIP1 plays a critical role in the prevention of cardiomyocyte cell cycle activation. Importantly, p21CIP1 appears to act as a suppressor on cardiomyocyte cell cycle by regulating the degradation of proliferating cell nuclear antigen (PCNA) rather than by its inhibitory effect on cyclin-dependent kinases.

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Engel FB, Hauck L, Boehm M, Nabel EG, Dietz R, von Harsdorf R (2003): p21CIP1 controls proliferating cell nuclear antigen protein level in adult cardiomyocytes. *Mol Cell Biol*, 23: 555-565

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Myocardial Nuclear Receptors in Heart Failure

Martin W. Bergmann (Helmholtz Fellow)

The group started in January 2001 focusing on the identification of new targets in heart failure treatment by characterizing the signal transduction pathways leading to cardiomyocyte hypertrophy and apoptosis. We study the role of specific transcription factors employing both isolated neonatal and adult cardiomyocytes as well as transgenic mice generated by Cre/lox techniques. Previous studies by other groups had implicated transcription factor NF-AT as an important downstream molecule integrating Ca^{2+} – induced signaling towards cardiomyocyte hypertrophy. Similar to NF-AT, the transcription factor NF- κ B was first identified for its prominent role in regulating inflammatory cytokines. However, NF- κ B was recently found to be important for B-cell proliferation in Hodgkin's disease and shown to be necessary for G-protein coupled receptor – induced cardiomyocyte hypertrophy *in vitro* as well as survival of cardiomyocytes. Similarly, the transcription factor cAMP response element binding protein (CREB) is essential for cAMP-induced cytokine release as well as cell proliferation and cardiomyocyte survival.

NF- κ B is a suitable target to improve left ventricular remodeling *in vivo*

We have characterized the signaling pathways induced by Angiotensin II (a well known hypertrophy stimulus) in adult cardiomyocytes. In addition, mice with heart-specific NF- κ B inhibition were generated by mating α -myosin heavy chain Cre-recombinase mice to loxP-I κ B Δ N mice in collaboration with R. Schmidt-Ullrich, group Scheidereit. 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- κ B inhibition. Gene chip analysis comparing adult cardiomyocytes with adenoviral overexpression of NF- κ B inhibitor I κ B Δ N to control virus transfected cells revealed a set of potential NF- κ B targets, which seem to be heart specific as a control of these genes by NF- κ B has not been described before. These target genes are currently validated by further experiments in

order to understand the mechanism of NF- κ B's influence on heart remodeling.

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- κ B activation, a role for CREB downstream of the PI3-kinase/AKT/GSK3 β signaling pathway was identified in these studies. Interestingly, GSK3 β did not alter CREB serine133 phosphorylation, the common endpoint of CREB stimulation regulating transactivation of CREB-responsive genes. Instead, GSK3 β regulated CREB DNA binding, possibly by a second phosphorylation at CREB serine 129.

Statins protect cardiomyocytes from apoptosis by inactivating GSK3 β

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 β pathway resulting in reduced apoptosis. Downstream of GSK3 β , the transcription factor β -catenin was stabilized as an effect of statin treatment.

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Cardiovascular Magnetic Resonance

Matthias G. Friedrich

Summary

The Cardiovascular Magnetic Resonance (CMR) group at the Franz-Volhard-Klinik has focused research on the in vivo assessment of functional and structural myocardial abnormalities related to infective inflammation and coronary heart disease.

Early tissue changes in myocardial infarction

Jeanette Schulz-Menger addresses issues related to myocardial hypertrophy and fibrotic scarring related to hypertrophic cardiomyopathy. She was able to show that contrast-enhanced magnetic resonance imaging detected infarct-related signal changes as early as 1 hour after acute myocardial infarction (AMI) in humans.

Magnetic relaxation properties of myocardial tissue

Daniel Messroghli focused his research on the development of new approaches to directly measure myocardial magnetic relaxation properties related to ischemia. He demonstrated that T1 mapping visualizes changes in the longitudinal relaxation time induced by acute myocardial infarction.

Assessment of myocardial water content

Hassan Abdel-Aty investigated myocardial edema related to acute myocardial infarction. He performed a study on the differential aspects of edema in acute and chronic settings and could verify the close correlation of myocardial edema to the stage of reperfused infarction.

Perfusion of the peri-infarct myocardial tissue

Andrew Taylor from the Baker Heart Research Institute, Melbourne, Australia spent one year in our group. He discovered that CMR detects impaired microvascular reperfusion in AMI patients despite successful infarct angioplasty, associated with a lack of recovery of wall motion.

Myocardial perfusion abnormalities

Nidal Al-Saadi investigated first-pass contrast-enhanced CMR to coronary angiography in a clinical setting and refined algorithms for the analysis of signal intensity changes in various setting.

Hb oxygenation changes in stress-induced myocardial ischemia

Matthias Friedrich focused his studies on the further development of using non-contrast CMR techniques to assess tissue oxygenation in acute myocardial ischemia. Using Blood-Oxygen-Level-Dependent magnetic resonance imaging (BOLD-MRI) to assess tissue oxygenation, he demonstrated that adenosine BOLD-MRI detects ischemia in myocardial segments related to severe coronary stenoses.

Future aspects

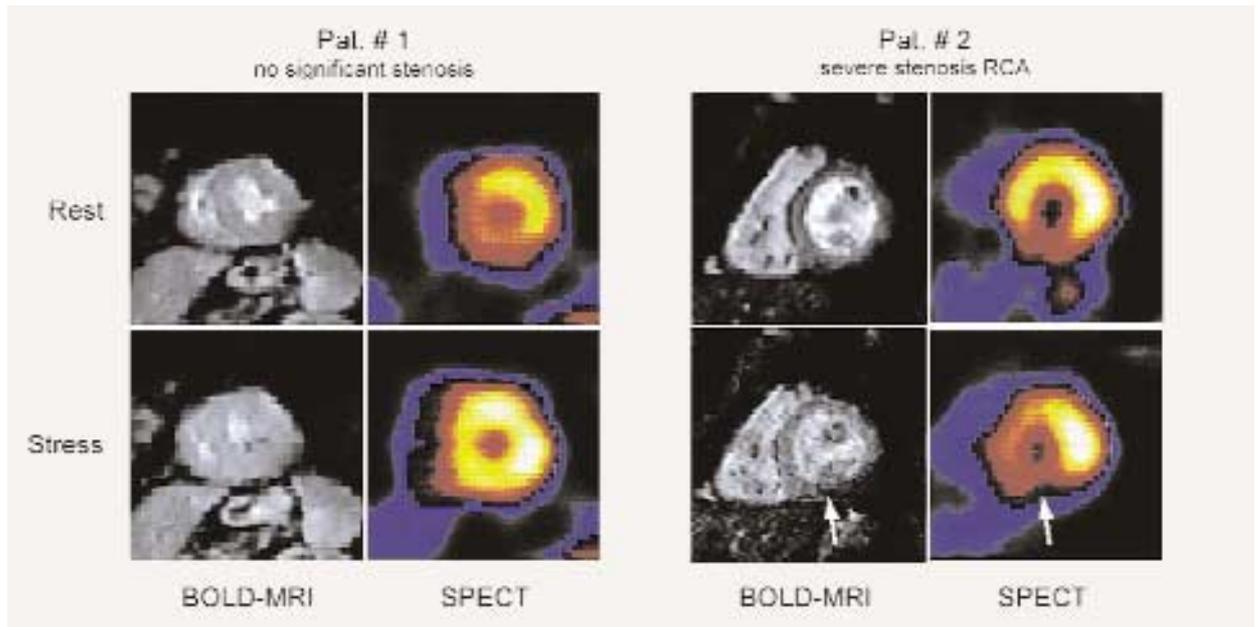
Matthias Friedrich has accepted a position of an Associate Professor for Cardiology and Director of the Cardiovascular MR Center at University of Calgary, Alberta, Canada. Andreas Kumar and Hassan Abdel-Aty will join him. Jeanette Schulz-Menger will assume responsibility for the group. A close relationship between Matthias Friedrich and the CMR department of the Franz-Volhard-Klinik is being established. Daniel Messroghli is currently spending a two-year stay at the CMR center of the University of Leeds and will return in January 2005 to continue his work (funded by the Marie-Curie foundation).

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BOLD-MRI images compared to conventional nuclear medicine (SPECT) images to visualize myocardial ischemia. Whereas the SPECT images reflect the accumulation of Thallium as a surrogate marker, BOLD-MRI directly reflects tissue oxygenation, thus provides molecular information in vivo.

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Friedrich MG, Niendorf T, Schulz-Menger J, Dietz R. (2003) Blood-Oxygen-Level-Dependent Magnetic Resonance Imaging in Patients with Stress-Induced Angina. *Circulation* 108:2219-2223.

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Molecular Muscle Physiology

Ingo L. Morano

Contractility of cardiac and smooth muscle is regulated by calcium ions (Ca^{2+}) that 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 (ryanodin receptors). Calcium ions activate both intracellular signalling pathways and contraction of the myofibrils. In cardiomyocytes, they activate 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+} form a complex with calmodulin that 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, ryanodine receptor, 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 level provides an opportunity to develop new therapies for the treatment of cardiac and smooth muscle dysfunction.

Understanding the molecular motor

Essential myosin light chain isoforms regulate human heart 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 ventricles of patients with congenital heart diseases and hypertrophic cardiomyopathy reexpressed

ALC-1, while MyHC isoenzymes did not change. Ventricular myosin associated with ALC-1 revealed a higher shortening velocity and rate of force development than normal cross-bridges without ALC-1. Maximal isometric force production per cross-sectional area as well as Ca^{2+} sensitivity of the force Ca^{2+} ratio were enhanced. The failing ventricles of patients with dilated cardiomyopathy, however, hardly expressed ALC-1. Therefore, an adenoviral vector containing the human ALC-1 (hALC-1) expression cassette (CASSETTE?) was developed for the upregulation of the hALC-1 in the cardiomyocytes of the failing human heart as a novel gene therapeutic approach.

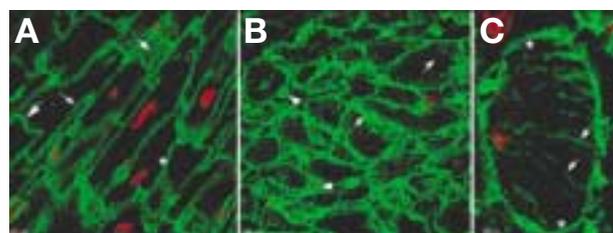
Regulation of smooth muscle contractility by recruitment of non-muscle myosin in an SM-MyHC knock-out model

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, the phasic contraction is generated by SM-MyHC recruitment while the sustained tonic contraction state can be produced by NM-MyHC activation. In addition, both contractile systems in smooth muscle are associated with different second messenger pathways. Both the SM-MyHC and NM-MyHC systems seem to be involved in electro-mechanical and pharmacomechanical coupling, respectively.

Understanding Ca^{2+} -handling proteins

L-type Ca^{2+} channels are multi-subunit proteins composed of the pore-forming $\alpha_1\text{C}$ subunit ($\text{Ca}_v1.2$) together with auxiliary subunits, α_2/δ , and β_2 . Alterations in the density or function of L-type Ca^{2+} channels have been implicated in a variety of cardiovascular diseases, including atrial fibrillation, ventricular hypertrophy, and heart failure. Activation of the beta-adrenergic receptor cascade markedly increases Ca^{2+} influx via protein kinase A (PKA)-dependent phosphorylation of the channel subunits: $\alpha_{1\text{C}}$, β_2 or still unrecognized associated proteins. In an attempt to define the molecular details of channel phosphorylation, we have identified the 700-kDa ahnak as tightly associated protein and prominent PKA target

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 disks (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).



in mammalian cardiomyocytes. Next, we characterized ahnak in normal human myocardium as a peripheral membrane protein associated with the cytoplasmic aspect of the plasma membrane including T-tubular structures (Fig. 1). Using truncated ahnak fragments, we demonstrated the presence of multiple β_2 -subunit interaction sites within ahnak's carboxy-terminal. This ahnak domain was also defined to be responsible for F-actin binding. Together, localization and interaction partner suggest a role of cardiac ahnak as sarcolemma support protein and as a linker between Ca^{2+} channels and subsarcolemmal cytoskeleton. Recent electrophysiological experiments demonstrated for the first time that carboxy-terminal ahnak fragments modulate specific aspects of Ca^{2+} channel gating (I_{CaL}) properties such as an increase in I_{CaL} amplitude and a slowing of inactivation. Hence, our results suggest that binding of the β_2 -subunit to the subsarcolemmal giant ahnak protein reprimers the α_{1C} - β_2 -subunit interaction and the relief of this inhibition increased the Ca^{2+} inward current. Ongoing studies include the generation of an ahnak-knock-out mouse model and a screening program to identify ahnak mutations in patients suffering from cardiomyopathies.

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Selected Publications

Lofgren M, Ekblad E, Morano I, Arner A (2003) Non-muscle myosin motor of smooth muscle. *J. Gen. Physiol.* 12: 301–310

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Cell Polarity During Development of *Drosophila* and Zebrafish

Salim Abdellilah-Seyfried

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 basolateral 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, 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 answer the following questions: 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? Our long-term interest is to understand how the cellular mechanisms controlling cell polarity shape our own bodies.

Cell polarity in zebrafish epithelial formation and organogenesis

The establishment and maintenance of polarity is an essential feature of eukaryotic cells. At the core of initiating and maintaining cellular polarity is the conserved Par protein complex that contains an atypical protein kinase C (aPKC) and the PDZ domain containing Par6 protein. The role of this protein complex in epithelial formation is best understood in *Drosophila* where Bazooka (*Drosophila* Par-3), Par-6, and aPKC localize to the apico-lateral membrane of embryonic epithelia, just apical and partially overlapping Armadillo (β -catenin) localization at the zonula adherens. The disruption of normal gene function causes epithelial defects, including loss of cellular polarity, loss of the zonula adherens, and changes in cell shape.

Large-scale screens for embryonic lethal mutations in zebrafish have isolated several mutations that affect epithelial integrity. One of these mutations, *heart and soul* (*aPKC ι*), affects early development and the formation of several polarized epithelia. Consistent with a conserved role of zebrafish aPKC ι , the protein is required for the formation and maintenance of adherens junctions in the polarized epithelia of the retina, neural tube, and digestive tract. During early stages of organogenesis, *heart and soul* appears to regulate the apical clustering and maintenance of adherens junctions. In addition to the epithelial defects, *heart and soul* affects the morphologies of the heart tube and the gut and some of its associated organs.

We have performed a functional analysis of aPKC ι by using a combination of antisense oligonucleotide morpholinos mediated gene “knock down” and coexpression of mutant *aPKC ι* mRNAs. This approach provides conclusive evidence that activity of the catalytic domain is essential in the context of vertebrate cell polarity and organ morphogenesis. Moreover, it identifies several highly conserved residues that have been implied in potential regulatory roles and demonstrates that they are indeed critical for aPKC ι function.

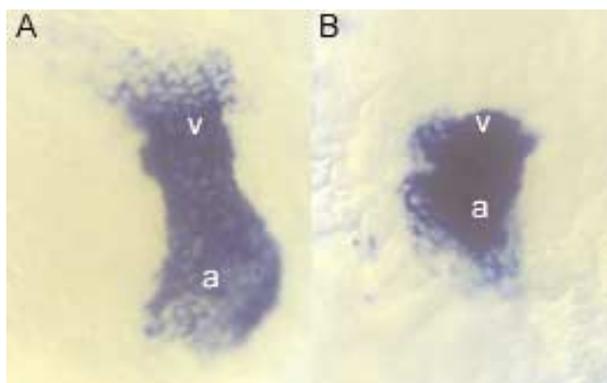
Research in our laboratory is currently directed towards identifying and characterizing the direct downstream targets of aPKC ι in the context of cell polarity and organ morphogenesis. Furthermore, we are involved in the cloning and characterization of other zebrafish mutations that affect cellular polarity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

Bazooka in cell migration

During the development of multicellular organisms, various types of directed cell migration occur that contribute to the development of different tissues. These include the migration of neural crest cells, hematopoietic stem cells, and germ cells. Gaining a better understanding of the mechanisms that govern normal cell motility and invasion is crucial for understanding development and may also contribute to understanding forms of aberrant cell invasion and migration of metastatic tumor cells.

Border cell migration during *Drosophila* oogenesis is one well-studied example of invasive and directed migration. Border cells are specified within the anterior follicular epithelium that surrounds the germ cells in each egg chamber, delaminate from the monolayer epithelium and, in a highly stereotyped fashion, invade the germ cell cluster. First, they undergo directed cell migration towards the oocyte and then turn dorsally.

We showed that wild-type *bazooka* (the *Drosophila* homolog of *par-3*) is required during cell invasion of epithelial follicle cells mutant for the tumor suppressor *discs large*. Clonal studies indicate that follicle cell Bazooka is a permissive factor during cell invasion, possibly by stabilizing adhesion



Heart morphogenesis defects in zebrafish epithelial mutant. **(A)** The zebrafish wild-type embryonic heart at 30 hours of development visualized with *cardiac myosin light chain 2* probe. The heart is an elongated two-chambered tube with an anterior atrium (a) and posterior ventricle (v). **(B)** In comparison, the heart tube is not elongated in *nagie oko* mutant embryos.

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between the invading somatic cells and their substrate, the germ line cells. Genetic epistasis experiments demonstrate that bazooka acts downstream of *discs large* in tumor cell invasion. In contrast, during the migration of border cells, Bazooka function is dispensable for cell invasion and motility, yet is required cell-autonomously in mediating cell adhesion within the migrating border cell cluster. Taken together, these studies reveal that Bazooka functions distinctly in different types of invasive behaviors of epithelial follicle cells, potentially by regulating adhesion between follicle cells or between follicle cells and their germ line substrate.

Selected Publications

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Cell Biology of Cardiovascular Diseases

M. Cristina Cardoso
(in collaboration with Heinrich Leonhardt)

Differentiation and proliferation of muscle cells

During terminal differentiation, striated muscle cells permanently withdraw from the cell cycle and become refractile to growth stimulation. We are interested in the molecular mechanisms regulating the establishment and maintenance of terminal differentiation and in devising ways to transiently reverse this state to achieve tissue regeneration. We have previously shown that this proliferation arrest is an actively maintained process that can be reversed upon transgenic expression of the simian virus 40 large T antigen (SV40 TAg). To avoid the hazards of gene therapy-based strategies, we are developing approaches to directly deliver the gene products (i.e., the proteins) to these cells. Taking advantage of the intercellular trafficking properties of the herpes simplex virus I VP22 protein, we have directly delivered SV40 TAg to striated muscle cells via fusion with VP22 and shown that it can stimulate cell proliferation. This protein transduction method allows for the simultaneous delivery of mixtures of regulatory proteins in a dose- and time-controlled fashion and it is easy to combine with the application of other compounds. We are presently optimizing this technology for tissue regeneration *in vivo* and for the expansion of differentiated stem cells (embryonic and adult) *in vitro*. In addition, in collaboration with the group of R. Kettritz (FVK), we are testing the applicability of this approach to other terminal differentiated cells, such as human neutrophils, which play an important role in the process of vasculitis.

Nuclear organization and genome replication

Although the nucleus 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 tag components combined with the ability to fluorescently tag proteins has revealed a different

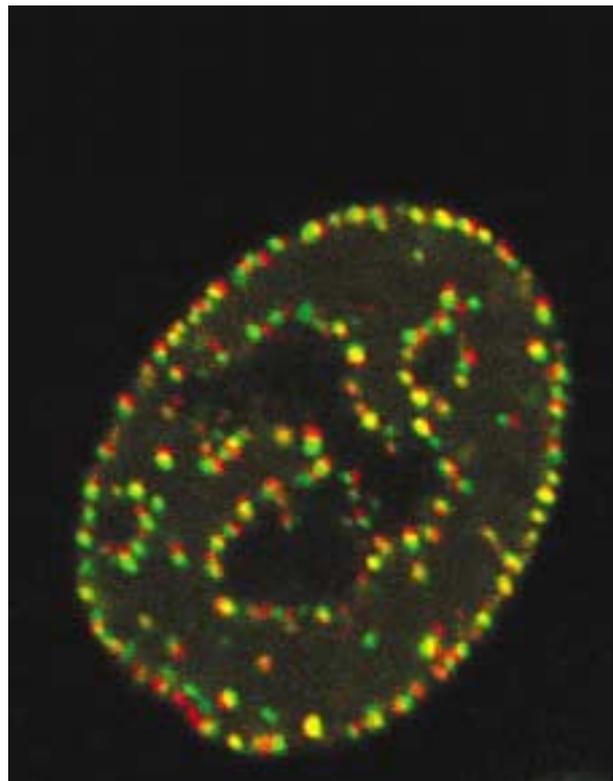
picture of the nucleus with many discrete and distinguishable subnuclear compartments involved in DNA or RNA metabolism. We are studying the coordination of the multiple enzymatic activities involved in the replication of the genome at every cell division cycle.

To study the dynamic regulation of these nuclear structures during the cell cycle *in vivo* and in real time, we have established an approach for the visualization of DNA replication in living cells using translational fusions of different replication factors to green (GFP) or red (DsRed) fluorescent proteins. Using high resolution time lapse microscopy, we could show that replication site patterns within the nucleus change in a characteristic manner throughout S phase.

To investigate whether the replication factors remain stably bound at replication foci or whether they are in constant exchange, we have used biochemical *in situ* extractions as well as fluorescence photobleaching techniques. Both experimental approaches showed that the PCNA (proliferating cell nuclear antigen) clamp was tightly bound at replication sites, showing only little exchange, if any. A comparison with another replication factor RPA (single-stranded DNA-binding protein) involved in the initiation of DNA replication showed that, while RPA exchanged in a time frame of seconds, PCNA showed virtually no turnover within several minutes. This has

Figure 1
Propagation of DNA replication

The image shows the progression of DNA replication in live mammalian cells. Spatio-temporal changes of DNA replication were followed with a GFP-PCNA fusion protein by time lapse microscopy. This time overlay shows the distribution of replication sites in green and their redistribution 15 minutes later in red. New replication sites (red) are de novo assembled at adjacent sites with replication proteins from the nucleoplasmic pool. "Reprinted from *Molecular Cell*, Vol. 10, Sporbert et al., 1355-1365, Copyright (2002) with permission from Elsevier".



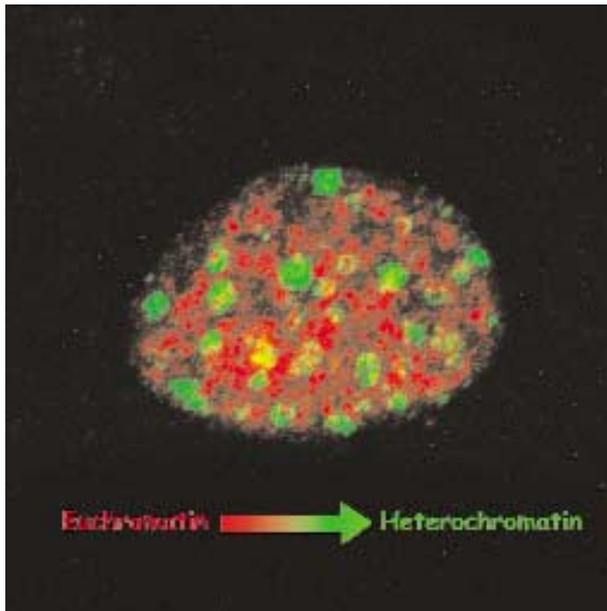


Figure 2

Genome replication program.

Mammalian cells expressing a GFP tagged DNA ligase I were pulse labeled for 10 minutes with the thymidine analog bromodeoxyuridine, followed by chase with 10-fold excess of thymidine. Three hours later, the cells were fixed and the incorporated nucleotide detected with specific antibodies (red) together with simultaneous detection of the GFP-DNA ligase I (green) localization. The image shows a projection of a z-stack of confocal images.

The early replicating chromatin (labeled with the nucleotide) consisting mostly of euchromatic regions is shown together with the late replicating chromatin (labeled three hours later with the tagged replication protein) comprising mostly constitutive heterochromatic centers. This spatial and temporal program of replication is followed at every cell cycle.

lead us to propose an alternative model for DNA replication, whereby the PCNA clamp stays bound throughout the synthesis of several Okazaki fragments. This could be achieved, as it was suggested for the DNA polymerase, by coupling the leading and lagging strand PCNA-polymerase complex together. We are currently testing this model by simultaneously measuring the on/off rate of PCNA and PCNA-binding replication factors in living cells. Since these fluorescence imaging techniques are based on averages of thousands of molecules, we, in collaboration with the groups of U. Kubitscheck and H. Leonhardt, are now tracing single molecules within living cell nuclei.

Replication of the mammalian genome starts at tens of thousands of origins that are activated at specific times during S phase raising the question of how this replication program is coordinated. Importantly, the spatio-temporal progression of DNA replication is inherited through consecutive cell division cycles. Our fluorescence photobleaching analyses showed that the transition from earlier to later replicons occurs by disassembly into a nucleoplasmic pool of rapidly diffusing subcomponents and reassembly at newly activated sites. A careful examination of the temporal and spatial assembly of new PCNA molecules by overlaying the images collected at consecutive times indicated that PCNA assembled in non-overlapping sites. These replication sites were in close proximity to earlier ones suggesting that activation of neighboring origins may occur by a domino effect possibly involving local changes in chromatin structure and accessibility. We are now trying to dissect the mechanism that under-

lies the ordered activation of later replication origins and sets the replication program.

Translation and replication of epigenetic information

Together with the genetic information, the epigenetic information is also duplicated and maintained over many cell generations. One of the essential epigenetic modifications in mammalian genomes is the methylation at position 5 of cytosines residues. We are analyzing different proteins involved in the maintenance and change of this epigenetic modification and their dynamic interaction with the replication machinery. Both the replication of genetic and epigenetic information are required for stable gene expression patterns.

We are approaching these questions via the identification and characterization of functional domains of the known DNA methyltransferases (Dnmt1, 2, 3a and 3b) and methyl-cytosine binding proteins (MeCP2, MBD1-4). We have recently found that Dnmt1 binds to the replication machinery during S phase via its interaction with PCNA and remains bound to centromeric heterochromatin during both G2 phase and M phase via a separate targeting sequence. Deletion of this sequence as well as its overexpression indicate an essential role in the methylation of centromeric repeat sequences and in chromatin stability and organization.

Mice carrying a hypomorphic Dnmt1 allele, which reduces Dnmt1 expression to 10% of wild type levels, exhibited genomic hypomethylation and developed aggressive tumors with a high incidence of chromosome 15 trisomy. These results provide a causal link between DNA hypomethylation and tumor formation, possibly by promoting genomic instability.

We are now investigating the role of other functional domains in vivo using transgenic animal approaches as well as testing the role in the maintenance of other epigenetic modifications. We are also investigating whether these functions are conserved during evolution. Furthermore, we are studying the role of methyl-cytosine binding proteins in the translation of epigenetic information.

Selected Publications

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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), and 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 confirmation of the receptors resulting in the agonist-like effect.

Autoantibodies in myocarditis and dilated cardiomyopathy (DCM)

The suggestion that the anti- β_1 -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 that in patients with these autoantibodies disappeared 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 -ad-

renoceptor 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. It was shown in a pilot study that the treatment of DCM patients with this specific adsorption results in improved cardiac function.

Autoantibodies in hypertension

Furthermore, we have investigated the role of autoantibodies in essential and therapy refractory hypertension. In the sera of patients with this disease, we detected autoantibodies directed against the α_1 -adrenoceptor. These autoantibodies recognize epitopes on the first or second extracellular loop of the α_1 -adrenergic receptor and act like α_1 -adrenergic agonists. In patients with hypertension refractory to therapy, more than 80% of the patients were antibody positive. In patients with malignant hypertension, those with acute vascular kidney rejection, and those with preeclampsia, we observed autoantibodies against the angiotensin II AT₁-receptor. In preeclamptic patients, this antibody is detectable after the 20th week of pregnancy and disappears after delivery. These agonist-like anti-AT₁-receptor antibodies induce formation of the transcription factors AP-1 and NF κ B and activate NADPH oxidase. These functional autoantibodies are found in more than 90% of preeclamptic women investigated and may play a role in elevating vascular resistance and promoting hypertension and cardiac hypertrophy in these patients.

Autoantibodies in Raynaud's syndrome

Raynaud's syndrome is characterized by a cold induced reduction of the blood flow into small vessels of the extremities. The pathogenesis of this disease is not fully understood. We have observed that the sera of patients with Raynaud's syndrome contain functional autoantibodies against protease-activated receptors (PAR). The antibodies react with the second extracellular loop of both the PAR-1 (thrombin receptor) and the PAR-2 (tryptase receptor) because the epitope of the antibodies on the receptor is identical for these receptor subtypes. Our investigations aim to elucidate the role of these autoantibodies in the development and/or maintenance of this disease.

Selected Publications

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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 (see also progress report by Ludwig Thierfelder). In striated muscle, titin forms a continuous filament system and serves as a template to assemble the sarcomere providing multiple binding sites as depicted below.

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.

Recent work has shown that even smooth muscle and non-muscle cells express titin or titin-like proteins, albeit at low levels compared to striated muscle. A plethora of non-muscle functions have been proposed for titin such as a role in chromosome condensation, chromosome segregation, or in the assembly of actin stress fibers but none of these has been demonstrated conclusively. So far, there is no comprehensive investigation of non-muscle and smooth muscle titin expression or splicing and conflicting data on its proposed function.

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.

Stretch signal

Titin is a unique molecule that contains elastic spring elements and a kinase domain, as well as 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 has remained unknown how the stretch signal is processed, i.e. how the mechanical stimulus stretch is converted into a biochemical signal.

To understand the stretch signaling pathway, we utilize mouse genetics, biomechanics, and signal transduction analysis to study the interplay of titin's elastic and catalytic regions and their regulation in a stretch-dependent fashion.

Smooth muscle and non-muscle titins

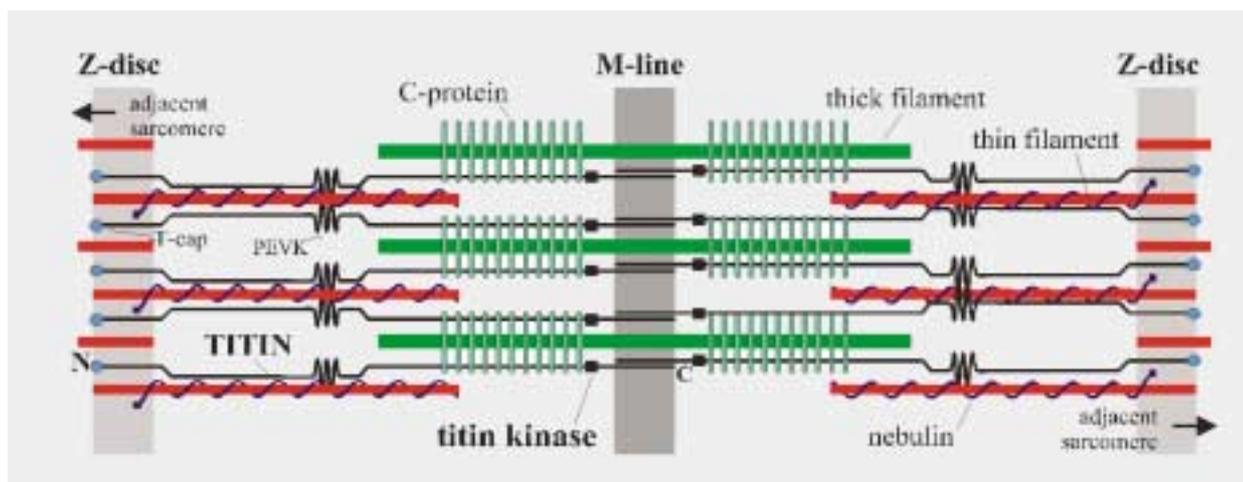
Only recently, the muscle protein titin has been proposed to perform non-muscle functions, following its localization to various cell compartments such as the chromosomes of *Drosophila* neuroblasts and the brush border of intestinal epithelial cells. Titin has been implicated in cytokinesis through 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. 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 (cytokinesis and chromosome segregation) to establish the role of titin in non-muscle cells.

Functional analysis of individual titin domains

To lay the groundwork for the *in vivo* analysis of titin's multiple signaling, elastic, and adaptor domains and their interplay in muscle as well as in non-muscle cells, we have started with the generation of 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 in muscle and in non-muscle cells.

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



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.

Selected Publications

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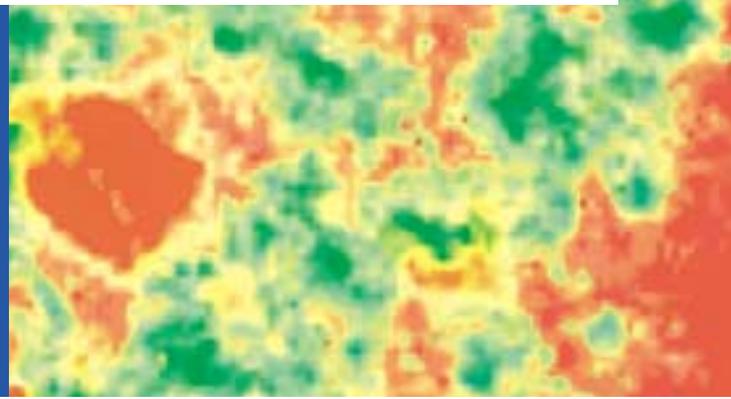
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Bioinformatics

Jens Reich
(in collaboration with Peer Bork)

Individual variation of the human genome and its role in lipid metabolism: genetic-epidemiological study of risk factors for arteriosclerosis

The lipid network group (J.R.) continued its participation in a joint study to evaluate the importance of single nucleotide polymorphisms (SNPs) and splicing variants in the human genome. This was combined with an application study involving 1500 healthy individuals recruited by Friedrich Luft at the Franz-Volhard-Clinic (FVK) in which 93 SNP variants at 13 relevant gene loci were measured together with 5 clinically important indicators of human lipoprotein metabolism. This is the first time that the individual genotype of common SNPs (i.e. > 3% population frequency in a German population sample) relevant to a metabolic pathway were correlated with the physiological level of the resultant phenotype.

Lipid traits are the major risk factor for arteriosclerosis and its severe complications (e.g., myocardial infarction, stroke, etc.). Physiologically valid phenotypic values (plasma levels of total cholesterol, TC; triglyceride, TG; low density lipoprotein, LDL, high density lipoprotein, HDL; and the important clinical risk factor LDL/HDL) were measured at the FVK under standardized conditions and SNP genotyping was done by Peter Nürnberg's MDC Genomic Mapping Centre. Earlier comparative studies on homozygotic and dizygotic twins (Busjahn & Luft) had established that a global heritability component of between 30 and 40% contributes to the individual lipid level in humans. As SNPs are the most common genetic variants, we wanted to learn which of them change the phenotype levels to a statistically significant extent. This genotype-phenotype correlation was to be integrated into a pathway model that we have developed on the basis of theoretical studies of metabolic models (H. Knoblach et al., 2000). The data obtained were subjected to an exhaustive mathematical-statistical analysis using advanced computer techniques.

The studied polymorphisms showed common variation in the sample. The allelic association of these SNPs (expressed as

score of linkage disequilibrium, LD) varied with their distance on the genome. The full genomic structure of all loci could be established for all gene loci by reference to the NCBI data base of the human genome. About one third of the SNPs were found in the coding region (non-synonymous and synonymous), one-third in the intron segments, and one-third in non-translated extragenic regions (promoters etc.). We achieved a full coverage of the genomic loci by around 12 common SNPs. All gene loci were thus represented by considerable, statistically highly significant linkage disequilibrium (LD).

These data are in agreement with population-genetic models for a fully outbred panmictic population in Germany and also with published data on other genome sections. Such a situation allows the LD mapping of functional gene alleles at small distances from the marker SNP. To this end, we established the SNP-haplotype structure of all loci by way of analysis of the family pedigrees of all subjects, since they were recruited as nuclear families in a systematic field working project. Haplotyping was achieved with a newly developed computer program (Rohde & Fürst, 2001) that permits, with high confidence, the establishment of the chromosomal phase of SNP positions when diploid genotype data from nuclear families are available, as was our case. It was found that, in all of the studied gene loci, only a few (4 to 5) haplotypes accounted for the genotype of about 80-90% of the population sample. This means that chromosomal haplotypes of common SNPs are very "old" genomic structures (i.e. many tens of thousands generations old, not dissolved in the whole population by extensive recombination) and may therefore be reliable markers of functional alleles that explain the variation and risk status of the phenotype. This warrants a genotype-phenotype association study if controlled for stratification into subpopulations.

The association was evaluated in combination with linkage tests according to modern models of biometric genetics, which partition the total variance into additive genetic, polygenic, and environmental components. Comparison of genotype-phenotype correlation between and within families enabled a control for stratification (sample inhomogeneity) effects.

We addressed the following questions:

- What is the degree of heritability of lipid traits in our sample ?
- What part of the genetic component is due to the candidate loci which we genotyped, and which complementary part is due to gene loci not measured?

- What is the contribution of individual candidate loci to the whole genetic variation ?

This analysis was done in the following stepwise way:

Comparing general variance and interfamilial covariance of the lipid values, we established that 38% of the variation of HDL and 26% of the variation of LDL could be explained as inherited (heritability estimate). Introduction of the SNP genotypes and/or SNP haplotypes into the analysis permitted the separation of the relative contribution of our genotyped loci as compared to the polygenic background. In looking at HDL and LDL, about 50% and 75% of the genetic variation factor, respectively, was attributed to our candidate gene loci. Thus, we could show that a major part of the genetic influence can be accounted for by our measured genotypes.

We devised a variance partition method that could estimate the contribution of individual gene variation to the phenotype variation. The figure shows the result for HDL and LDL. In the case of HDL, the loci of hepatic lipase, CETP and ABCA1 are the main contributors, whereas ApoE, CETP, and hepatic lipase are the main factors responsible for the variation in the LDL phenotype.

The results (submitted) are a “proof of principle” of the “common-variant-explains-common-trait”-hypothesis (Collins & Chakravarti) for complex pathways. This is of high relevance for the prediction of the genetic contribution to the risk status of individuals. At present, we are exploring the possible application of the results for SNP diagnostics in lipid-relevant genes, with special emphasis on gender and age factors. There are a considerable number of haplotypes that allow a prediction of the risk or protection status of subjects with apparently normal cholesterol values with advancing age.

In parallel with these studies, we investigated the importance of splicing variants in the human genome, based on genomic EST date bases, with special emphasis on cancer tissues. We assembled a splice site database which has been published in the internet and in peer-reviewed papers (H. Pospisil). The possible importance of splice variants as markers or prognostic predictors of colon cancer was studied in collaboration with the Robert-Rössle-Klinik (P. Schlag, W. Kemmner).

The haplotype study group (K. Rohde) developed an entropy measure for linkage disequilibrium over multilocus haplotype blocks (M. Nothnagel) and studied the association of genetic traits to SNP haplotypes using an EM algorithm with Markov Chain Monte Carlo Techniques.

Selected Publications

Rohde, K. and Fürst, R. (2003). Association of Genetic Traits to Estimated Haplotypes from SNP Genotypes Using EM Algorithm and Markov Chain Monte Carlo Techniques. *Human Heredity* 56, 41-47.

Knoblauch, H., Bauerfeind, A., Krähenbühl, Ch., Daury, A., Rohde, K., Bejanin, St., Esseoux, L., Schuster, H., Luft, F.C. and Reich, J.G. (2002). Common haplotypes in five genes influence genetic variance of LDL and HDL cholesterol in the general population. *Human Molecular Genetics* 11, 1477-1485.

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Locuswise contribution of lipid-relevant gene loci to the genetic variance of LDL and HDL variance in the German population. Results are obtained from a multiple association analysis considering SNP-haplotypes. Panel a: LDL; Panel b: HDL.

(APOE - apoprotein E; APOB - apoprotein B; LDLR - LDL receptor; LIPC - hepatic lipase; CETP - cholesteryl ester transfer protein; ABCA1 - ABC transporter A1; LPL - lipoprotein lipase)

Fig 1. a) LDL-cholesterol

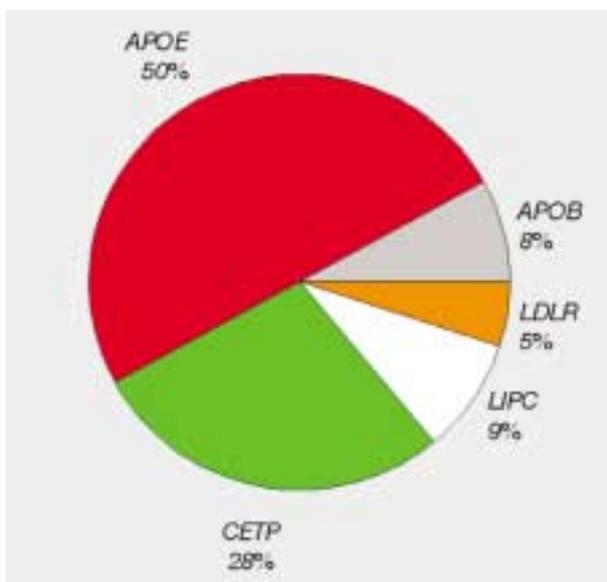
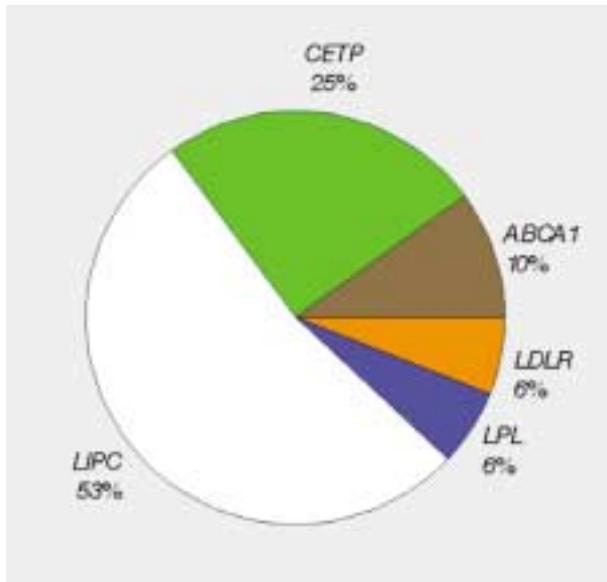


Fig 1. b) HDL-cholesterol



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Gene Mapping and Identification in Monogenic and Multifactorial Diseases

Peter Nürnberg

Positional cloning is now widely used for the identification of gene defects that underlie inherited diseases. A necessary first step for positional cloning is the mapping of the gene locus that co-segregates within families with a particular disease or trait, which allows the allocation of a specific chromosomal position to the responsible gene. Although mapping was initially developed for monogenic traits, it has now become the most widely used strategy to locate genetic factors involved in the aetiology of multifactorial diseases. The most powerful technique currently available is linkage analysis with highly polymorphic microsatellite markers, which involves an examination of the entire genome with a set of evenly spaced markers. This type of study is usually referred to as whole genome scan.

The Gene Mapping Centre is a specialised laboratory for such high-throughput genotyping for gene mapping of monogenic as well as multifactorial diseases. We have developed various sets of well-established markers including both microsatellites and single-nucleotide polymorphisms (SNPs). Different marker densities are used to accommodate the requirements of special study designs. The laboratory is mainly funded through grants from the German Federal Ministry of Education and Research (BMBF). Since 2001, we have participated in the National Genome Research Network (NGFN) as one of the major core facilities. Additional funding is provided from research grants of the DFG and through a strategy fund project (Genetics of Complex Diseases) from the Helmholtz Society of National Research Centres. The laboratory is open for mapping projects of other groups from Germany and abroad.

Mapping of multifactorial diseases

The main focus of the Gene Mapping Centre is mapping of genetic factors in multifactorial diseases. This type of study involves the analysis of large numbers of phenotypically well characterised families. Hundreds of markers are used for genotyping and sophisticated biostatistical analyses are

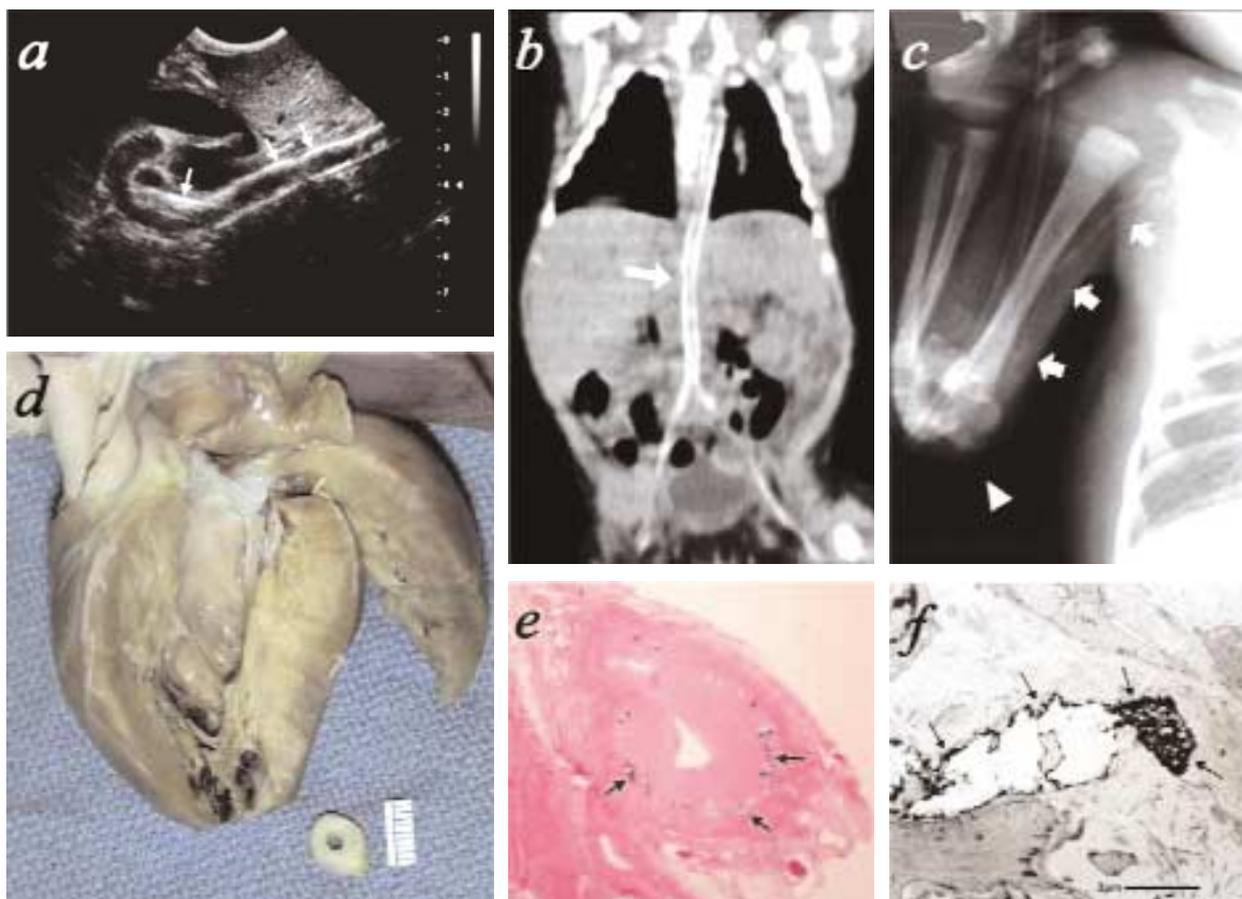
subsequently required to identify genetic loci that contribute to a complex disease. For this purpose, we have established the necessary techniques and emphasised automation of the experimental procedures. Our annual capacity is currently about 4,000,000 high-quality genotypes and is planned for expansion. Mapping is now mainly based upon genotyping of SNPs. Additionally, the laboratory is equipped for the large-scale analysis of highly informative microsatellite markers. Six scientists are involved in project management, genotyping, and technology development. Two scientists concentrate on laboratory information management (LIM) which involves the integration of genotype and phenotype data and the processing of these data for biostatistical analyses. This is done in close collaboration with the bioinformatics group (Dr. K. Rohde) and the University of Bonn (Prof. T. Wienker) who also manage the data analysis.

In a European collaborative study on the genetics of atopic dermatitis, a large number of families with two or more affected siblings were collected. A major susceptibility locus identified on chromosome 3q21 as well as further candidate regions are being investigated in these subjects. In a second European collaborative study, families are being investigated to identify genetic factors for susceptibility to common idiopathic generalised epilepsies (IGE). A novel IGE susceptibility locus on chromosome 3q26 and suggestive loci on chromosomes 14q23 and 2q36.1 are currently being pursued further with refined mapping and testing of positional candidate genes. Thus, we expect to gain important insights into the aetiology of both disease groups.

The identification of risk factors for complex traits is often facilitated by the analysis of pedigrees from isolated populations and takes advantage of the restricted genetic heterogeneity in these populations. Ongoing studies include genotyping of a study on the genetic factors in hypertension in collaboration with the Franz-Volhard Clinic in Berlin-Buch (Knoblauch et al. in press). Moreover, a locus for essential (primary) hypertension was mapped to chromosome 12p in a Chinese pedigree in collaboration with the MDC research group for Experimental Genetics of Cardiovascular Diseases (Gong et al. 2003). This locus appears to be of relevance for the identification of mechanisms leading to primary hypertension and of factors for cardiovascular morbidity and mortality. Running costs for all the studies are funded through additional external grants.

Mapping of monogenic diseases

In contrast to multifactorial diseases, mapping of monogenic traits requires less genotyping effort. Usually, it is sufficient to analyse 30 probands or less. The statistical evaluation is different and often requires skilled interpretation, for instance haplotyping. In the eight years of the existence of the lab, more than 60 monogenic traits have been mapped in humans. For several of these the underlying gene defect has been identified, completing the process of positional cloning. The identification of mutations in *ANKH*, the human ortholog of the mouse progressive ankylosis gene, in patients with cranio-metaphyseal dysplasia (CMD) led to the investigation of so-called 'idiopathic' infantile arterial calcification (see figure).



Manifestations of ILAC. **a**, Echocardiogram (parasternal view) of the affected girl from family 3 at age 5 days showing bright echogenic walls of the aortic arch and descending aorta (arrows), consistent with aortic calcifications. **b**, Native coronal CT scan of the thorax, abdomen and pelvis of the affected boy from family 4 at age 11 days. Note calcification of the entire aorta (arrow) and iliac arteries bilaterally. **c**, Radiogram of the right arm of the affected girl from family 5 at age 10 days. Note extensive peri-articular calcification of the elbow (arrowhead) and calcified brachial artery (arrows). **d**, Section through the left ventricle of the heart of patient 6, who died at age 45 days. Note hemorrhagic ischemic necrosis at the apex of the left ventricle and pallor of the subendocardial wall representing bland coagulative ischemic necrosis. A cross section of the aorta laid adjacent to the apex shows striking mural thickening and luminal narrowing. **e**, Cross section through the epicardial left anterior descending coronary artery from the same patient showing extensive calcifications of the internal elastic lamina (arrows) and massive myointimal proliferation (haematoxylin-eosin, 20X). **f**, Electron micrograph of the mineralized internal elastic lamina of the carotid artery from the same patient showing mineral deposition (arrows) with extracellular collagen fibrils and elastic fibers.

The genetic basis for the disease was disclosed by identifying underlying mutations in *ENPP1*, the gene for ectonucleotide pyrophosphatase/phosphodiesterase 1 (Rutsch et al. 2003). The gene product is a transmembrane glycoprotein involved in the metabolism of inorganic pyrophosphate (PP_i). The work on the further characterisation of CMD and related disorders is funded within the SFB 577 (Molecular Basis of Clinical Variability in Mendelian Disorders). Understanding this group of monogenic diseases will provide us with novel insights into more general processes controlling bone density and may offer new approaches to the therapy of osteoporosis.

Another focus of the group is the molecular characterisation of hereditary skin diseases. Autosomal recessive congenital ichthyosis, a severe genodermatosis characterised by scaling of the skin on the complete body surface, is both clinically and genetically heterogeneous. The identification of further loci and the molecular characterisation of different pathways leading to the disease is a goal of a new BMBF-funded network (Network Rare Diseases: Ichthyoses and Related Dis-

orders of Keratinisation). Mal de Meleda, an autosomal recessive palmoplantar keratoderma, was named after an island on the coast of Croatia where the high frequency of the disease is based on a founder effect. However, mutations in the underlying gene, which is related to cytotoxins of snakes, show clear allelic heterogeneity (Eckl et al. 2003). The molecular characterisation of such rare keratinisation disorders gives insight into processes of epidermal differentiation and provides models for more frequent, complex genetic skin diseases.

Several pedigrees are being investigated with various hereditary types of cardiomyopathy including dilated and hypertrophic forms. Because of the heterogeneity of these disorders, the study is based on linkage analysis in single pedigrees and candidate gene analysis. In the gene *CRP3* that encodes muscle LIM protein, mutations were found in patients with familial hypertrophic cardiomyopathy but not with dilated cardiomyopathy (Geier et al. 2003). *CRP3* was chosen as a candidate gene because muscle LIM protein deficient mice exhibited a disruption of cardiac cytoarchitectural

organisation and developed a marked cardiac hypertrophy reaction and dilated cardiomyopathy. Dealing with phenotypes comprising extensive heterogeneity is also relevant to various diseases associated with renal anomalies. Several loci for nephronophthisis and related nephritic disorders were mapped in the Centre (Ruf et al. 2003).

In addition, we have continued mapping monogenic traits in animal models, mainly mice and rats. Several spontaneous and ENU induced mutants were mapped, and in many cases, the underlying mutations were identified. The majority of the projects from external laboratories originated from Germany but also from England, France, The Netherlands, Canada, the U.S., the Republic of South Africa, the United Arab Emirates, Australia, and other countries.

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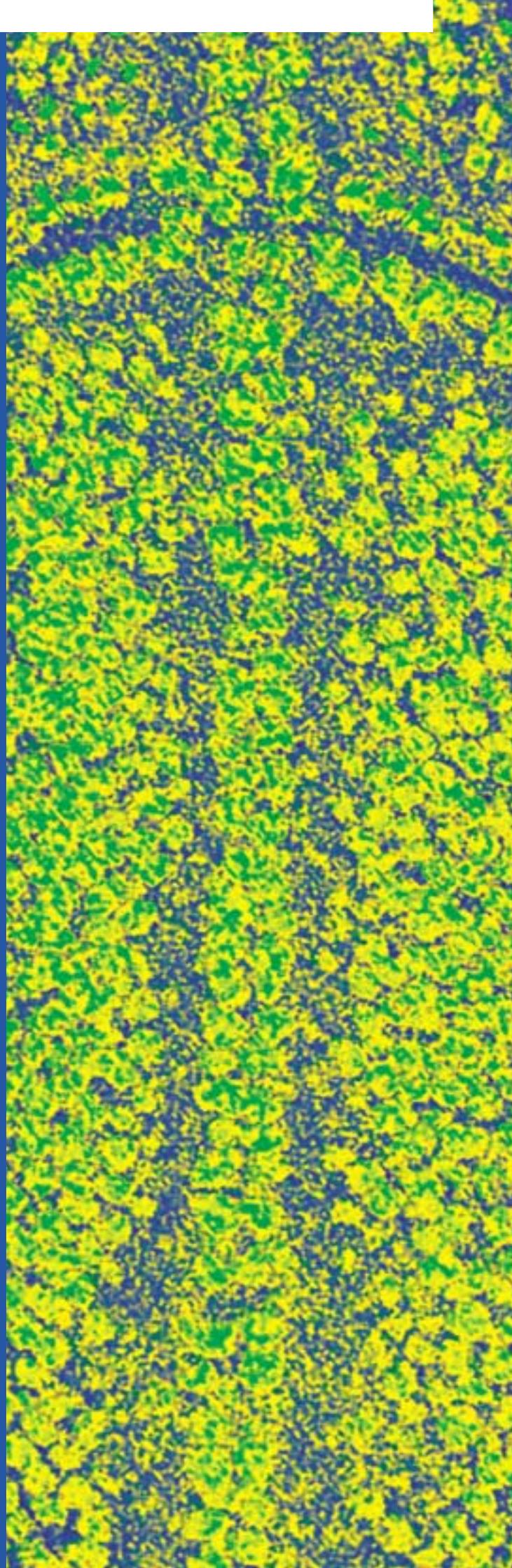
* part of the period reported

Cancer Research

Signalling Pathways, Cell Biology,
and Cancer
Coordinator: Walter Birchmeier

Structural and Functional Genomics
Coordinator: Udo Heinemann

Tumor Immunology
Coordinator: Martin Lipp



Cancer Research Program

Walter Birchmeier
Achim Leutz
Udo Heinemann
Martin Lipp

Cancer is a collective term for 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 for 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 of 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/Humboldt University in 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

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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 „Tumor-Suppressor“-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 Tumorummunologie tätig sind. Diese Untersuchungen werden in enger Zusammenarbeit mit den klinisch orientierten Gruppen der Robert-Roessle-Krebsklinik der Charité/Humboldt-Universität 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.

Signaling Pathways, Cell Biology, and Cancer

Epithelial morphogenesis and differentiation have been analyzed by defining the adhesive and signaling capacities of the E-cadherin/catenin/Wnt system (W. Birchmeier). In addition, the role of the scatter factor/hepatocyte growth factor and its receptor, the Met tyrosine kinase, has been examined in the morphogenesis of epithelial cells. Several components of the Wnt- and Met pathways have been found to be mutated in a variety of human tumors. Several new members of these pathways have been discovered in this laboratory (for example, Lef-1, Conductin, Diversin, Gab1, Dok's, and Hakai). The function of these novel proteins is currently investigated by genetic means in mice and in human tumors. Mouse models are generated which reflect particular genetic alterations found in human cancers. It was recently found that β -catenin, a component of the Wnt pathway, is a key regulator of the formation of the apical ectodermal ridge (AER) and of the dorsal-ventral axis of vertebrate limbs by conditional gene deletion in mice. It was demonstrated that β -catenin acts downstream of the BMP receptor IA in AER induction, but upstream or parallel to this receptor in dorsal-ventral patterning by generation of compound mutants (Soshnikova et al., *Genes & Development* 17, 1963-1968, 2003).

The reason why cancers of similar stage and histomorphological characteristics exhibit variable clinical outcomes remains unknown. The genetic reasons underlying differences in the aggressiveness of local tumors growth, in the ability of tumors to metastasize or in the susceptibility to chemo- and radiotherapy also remain largely unknown. Microarray and differential display techniques have been used (P. Schlag/W. Birchmeier) to identify genes that are responsible for variable tumor behavior (of primarily colorectal cancers, breast cancers, and sarcomas). The Robert-Roessle Clinic at the MDC has a large tissue and serum bank, consisting of more than 10,000 tissue samples derived from human primary tumors, their metastases, and from corresponding normal tissues. This bank forms the backbone of the described research programme.

The emergence of leukemia is connected to the deregulation and mutation of genes that encode critical regulators involved in hematopoietic stem cell biology and in blood cell differentiation. Transcription factors of the C/EBP family, c-Myb, and Scl/Tal1 balance the finely tuned system of stem cell self-renewal (A. Leutz). Mutations in these genes may cause leukemic conversion in these cells. It was found recently that the mRNAs for C/EBP α , C/EBP β , and Scl/Tal1 give rise to alternatively initiated protein isoforms with largely different functions in growth control and cell differentiation. Alternatively initiated Scl/Tal1 proteins may induce differentiation towards various blood cell lineages (Calkhoven et al., *Genes & Development*, 17, 959-964, 2003). Furthermore, the function of C/EBP β is regulated by the oncogenic ras signaling pathway. Ras signals determine whether C/EBP β acts as an activator or as a repressor of genes by regulating the interaction of C/EBP β with active and/or repressive "Mediator" complexes. Mediators are large multi-protein complexes found in all eukaryotes that link transcription factors to the basic transcription machinery (Mo et al., *Molecular Cell*, 13, 1-10, 2004).

Signalwege, Zellbiologie und Krebs

Morphogenese und Differenzierung von Epithelzellen wurden anhand der Haft- und Signaleigenschaften des E-Cadherin/Catenin/Wnt-Systems analysiert (W. Birchmeier). Außerdem wurde die Rolle des Scatterfaktors/Hepatozyten-Wachstumsfaktors und seines Rezeptors, der Met-Tyrosinkinase, in der Morphogenese von Epithelzellen analysiert. In einer Vielzahl menschlicher Tumore wurden Mutationen mehrerer Komponenten der Wnt- und Met-Signalwege festgestellt. Mehrere neue Komponenten dieser Signalwege (wie z.B. Lef-1, Conductin, Diversin, Gab1, Dok's und Hakai) sind in diesem Labor ermittelt worden. Die Funktionsweise dieser neu entdeckten Proteine wird derzeit mit genetischen Mitteln in Mäusen und in menschlichen Tumoren untersucht. Mausmodelle werden hergestellt, die besondere, in menschlichen Tumoren vorgefundene genetische Veränderungen widerspiegeln. So wurde kürzlich durch die konditionelle Inaktivierung von Genen in Mäusen festgestellt, dass β -Catenin, eine Komponente des Wnt-Signalweges, ein Schlüsselregulator für die Bildung der embryonalen apikal-ektodermalen Leiste (AER) und der dorsal-ventralen Achse der Gliedmaßen ist. Es konnte belegt werden, dass β -Catenin in der AER-Induktion unterhalb des BMP-Rezeptors IA, in der dorsal-ventralen Musterung aber oberhalb dieses Rezeptors aktiv ist (Soshnikova et al., *Genes & Development* 17, 1963-1968, 2003).

Die Ursache dafür, dass verschiedene Krebsformen ähnlicher Stadien und histomorphologischer Eigenschaften variable klinische Resultate zeigen, ist nicht geklärt. Auch die genetischen Gründe, die für Unterschiede in der Aggressivität des lokalen Tumorwachstums, der Fähigkeit von Tumoren zur Metastasenbildung oder ihrer Empfänglichkeit gegenüber Chemo- und Radiotherapie ausschlaggebend sind, sind weitestgehend unbekannt. Um Gene zu identifizieren, die für variables Tumorverhalten verantwortlich sind, wurden Mikroarray- und „Differenzialdisplay“-Verfahren bei kolokleralen Tumoren, Brustkrebs und Sarkomen angewandt (P. Schlag/W. Birchmeier). Die Robert-Roessle-Klinik am MDC besitzt eine große Gewebe- und Serumbank mit über 10.000 Gewebeproben, die von menschlichen Primärtumoren, ihren Metastasen und von entsprechenden Normalgeweben stammen.

Das Auftreten von Leukämie steht in Verbindung mit der Deregulation und Mutation von Genen, deren Produkte die Blutzellbildung und Blutzelldifferenzierung bestimmen. Die Transkriptionsfaktoren der C/EBP-Familie sowie c-Myb und Scl/Tal1 kontrollieren die fein abgestimmte Balance zwischen Stammzellen und Zelldifferenzierung (A. Leutz). Mutationen in diesen Genen können zu Leukämien führen. Es wurde kürzlich festgestellt, dass die Expression der C/EBP α -, C/EBP β - und Scl/Tal1-Proteine durch differentielle Initiation ihrer mRNA-Translation reguliert sind. Alternativ initiierte Scl/Tal1-Proteine können die Differenzierung in verschiedene Blutzelllinien hervorrufen (Calkhoven et al., *Genes & Development*, 17, 959-964, 2003). Zudem wurde erkannt, dass die Funktion des C/EBP β , welches in vielen Tumoren exprimiert ist, durch das ras-Tumorprotein reguliert wird. Ras-Signale bestimmen, ob C/EBP β als Aktivator oder Repressor von Genen agiert, indem sie die Interaktion des C/EBP β mit aktiven oder mit repressiven „Mediator“-Kom-

An example of signal-dependent gene regulation with an extensive medical relevance is the nuclear factor κ B (NF- κ B) transcription factor family (C. Scheidereit). NF- κ B is essential for innate and adaptive immunity, but also plays important roles in cell proliferation, programmed cell death, and in early embryogenesis. NF- κ B has been found to contribute to the development of diseases such as cancer, various inflammatory disorders, and skin diseases. NF- κ B activation pathways are analyzed biochemically and in mouse models. Current emphasis has been given to the genome-wide identification of NF- κ B target genes and to the identification of cross-talk with other transcription factors and signaling pathways (Hinz, M., et al., *J. Exp. Med.* 196, 605-17, 2002). Mouse models for human disease have been generated. A crosstalk between the NF- κ B pathway and JUN proteins in lymphomagenesis has recently been established. c-JUN was shown to be activated by autonomous pathways, with NF- κ B playing a role in determining the composition of the AP-1 subunit by upregulating JunB. AP-1 cooperates with NF- κ B at target gene promoters. c-Jun and JunB overexpression is observed in the great majority of classical Hodgkin's tumors, and to a lesser extent in anaplastic large cell lymphomas but not in other B- or T-cell malignancies (Krappmann et al., *EMBO J.* 21, 4104-4113, 2002).

Rapid and specific proteolysis via the ubiquitin-proteasome pathway is a key step in many regulatory processes and also important for the elimination of mis-folded proteins. Defects in this pathway are associated with the development of disease including cancer and viral infections (T. Sommer). Components and underlying principles of the evolutionary highly-conserved ubiquitin system are being studied. Research focuses mainly on the compartmentalized functions of the system, which bring together the fields of specific proteolysis and of intracellular protein transport and secretion. Endoplasmic reticulum (ER) associated protein degradation by the ubiquitin proteasome system requires dislocation of the proteolytic substrates from the ER into the cytosol. Recent analyses revealed that the AAA ATPases of the 26S proteasome are not directly involved in exporting an ER-luminal substrate. Instead, a related AAA ATPase-complex, Cdc48p/p97, plays a crucial role in ER associated protein degradation upstream of the proteasome (Jarosch, E., *Nature Cell Biol.* 4, 134-139, 2002).

Tight control over initiation of DNA replication is of central importance for cell proliferation to ensure normal development and differentiation. It is still not fully understood how particular DNA sequences regulate this process, whereas the function of proteins involved in this process in higher eukaryotes have begun to emerge. Aspects of replication initiation in both *Drosophila* and in mammalian cells are being studied (M. Gossen).

The Sleeping Beauty transposon shows high transpositional activity in cells of vertebrate species (Z. Ivics). Thus, this transposable element is a useful tool for genetic engineering and gene discovery in vertebrates, as well as for the dissection of molecular mechanisms of transposon-host cell interactions. Depending on the insertion site, inducible transcription from promoters inside the transposon will overexpress and/or ectopically express endogenous genes or parts of genes. This

plexen regulieren. Mediator-Komplexe sind große Multiproteinkomplexe, die in allen Eukaryoten vorkommen und Transkriptionsfaktoren mit der basalen Transkriptionsmaschinerie verknüpfen (Mo et al., *Molecular Cell*, 13, 1-10, 2004).

Die Familie des Kernfaktors NF- κ B ist ein Beispiel für die signalabhängige Genregulierung einer Transkriptionsfaktor-Familie, die von weitreichender medizinischer Bedeutung ist. NF- κ B spielt eine bedeutende Rolle für die angeborene und adaptive Immunität, hat jedoch auch erheblichen Einfluss auf die Zellproliferation, den programmierten Zelltod und die frühe Embryogenese (C. Scheidereit). NF- κ B trägt nachweislich zur Entwicklung von Krankheiten wie der Tumorgenese, verschiedenen Entzündungsleiden und Hautkrankheiten bei. Die NF- κ B-Aktivierungswege werden biochemisch und in Mausmodellen analysiert. Besonderer Nachdruck wurde in letzter Zeit auf die genomweite Identifizierung von NF- κ B-Zielgenen und den Nachweis des Zusammenspiels mit anderen Transkriptionsfaktoren und Signalwegen gelegt (Hinz, M., et al., *J. Exp. Med.* 196, 605-17, 2002). Ein Zusammenwirken zwischen NF- κ B- und den JUN-Proteinen in der Lymphomagenese wurde kürzlich nachgewiesen. Dabei ergab es sich, dass c-JUN von autonomen Signalwegen aktiviert wird, wobei NF- κ B eine Rolle bei der Bestimmung der Zusammensetzung der AP-1-Untereinheit spielt, indem es JunB hochreguliert. AP-1 kooperiert zudem mit NF- κ B an Zielgen-Promotern. Eine Überexpression von c-Jun und JunB wird in der überwiegenden Mehrheit klassischer Hodgkin-Tumore und in geringerem Maße in anaplastischen, großzelligen Lymphomen, jedoch nicht in anderen B- oder T-Zelltumoren beobachtet (Krappmann et al., *EMBO J.* 21, 4104-4113, 2002).

Die schnelle und spezifische Proteolyse über das Ubiquitin-Proteasom ist ein Schlüsselschritt für viele Regulationsprozesse und von zentraler Bedeutung für die Eliminierung fehlgefalteter Proteine. Defekte in diesem Signalweg sind für die Entwicklung von Krankheiten einschließlich Krebs und Virusinfektionen verantwortlich (T. Sommer). Bestandteile und grundlegende Prinzipien des evolutionär hochkonservierten Ubiquitin-Systems werden derzeit untersucht. Die Forschung konzentriert sich hauptsächlich auf die kompartimentalisierten Funktionen des Systems, die die Bereiche der spezifischen Proteolyse, des intrazellulären Proteintransports und der Sekretion verknüpfen. Die durch das Ubiquitin-Proteasomsystem hervorgerufene Proteindegradation steht mit dem endoplasmatischen Retikulum (ER) in einem engen Zusammenspiel und erfordert eine Dislokation der proteolytischen Substrate aus dem ER in das Zytosol. Neuerliche Analysen haben ergeben, dass die AAA ATPasen des 26S Proteasoms nicht direkt am Export eines ER-Lumenssubstrats beteiligt sind. Stattdessen spielt ein verwandter AAAATPase-Komplex, Cdc48p/p97, der oberhalb des Proteasoms angreift eine entscheidende Rolle in der ER-assoziierten Proteindegradation (Jarosch, E., *Nature Cell Biol.* 4, 134-139, 2002).

Für die normale Entwicklung von Zellen und für die Regulation der Zellproliferation und Differenzierung ist eine strenge Kontrolle der Initiierung der DNA-Replikation von zentraler Bedeutung. Es ist bis heute nicht vollständig bekannt, wie bestimmte DNA-Sequenzen diesen Prozess regulieren, während

can be exploited for the discovery of novel oncogenes and tumor suppressors using dominant screens both in tissue culture and in living organisms. As transposition inherently involves the generation of DNA damage, the Sleeping Beauty element can serve as an experimental system to study the basic molecular mechanisms involved in DNA repair. It has recently been shown that non-homologous end-joining and homologous recombinational repair pathways both contribute to the repair of transposition-induced DNA damage. Thus, important similarities as well as differences exist between cellular responses to V(D)J recombination, retroviral integration, and DNA transposition (Izsvak et al., Mol. Cell 9, 147-156, 2004).

Structural and Functional Genomics

The systematic analysis of three-dimensional protein structures, commonly known as structural genomics, has been made possible by the completion of the human genome project and through technical developments in recombinant protein production (U. Heinemann). A consortium of research institutions based in the Berlin-Brandenburg area has established facilities and a technical approach that allow the structure determination of human proteins by NMR spectroscopy and X-ray crystallography at a vastly accelerated rate and with greater accuracy (Heinemann et al., Acc. Chem. Res. 36, 157-163, 2003). The human protein structures solved so far include those of the translational inhibitor p14.5 (a protein that is downregulated in liver and kidney tumors), gankyrin (the product of a gene linked to hepatocellular carcinoma that has been described to physically interact with the retinoblastoma protein, Rb), the cyclin-dependent protein kinases 4 and 6, the 26S proteasome, and Bet3p, a central subunit of the vesicle-tethering TRAPP complex located at the cis-Golgi membrane.

Computer simulations of macromolecular structures provide insights into their conformational transitions and dynamics—information which cannot be provided by experimental structure determination approaches (H. Sklenar). A new Monte Carlo simulation algorithm, that permits prediction of sequence-dependent structures of DNA and the study of DNA-ligand interactions, has been implemented and tested. Zinc-finger proteins were identified and annotated for the completed sequenced genomes of *Drosophila melanogaster* and *Arabidopsis thaliana* in genome-wide computer searches (S. Böhm). A novel zinc-finger-associated domain (ZAD) restricted to insects was identified (Chung et al., EMBO Rep. 3, 1158-1162, 2002).

Breast or ovarian cancer usually arises sporadically but a fraction (~10%) of breast-cancer cases are heritable and are caused by mutations in the tumor-suppressor genes BRCA1 or BRCA2. Additional high- and low-penetrance genes linked to breast cancer are currently being identified by linkage analysis in high-risk families and in association studies involving large cohorts of patients (S. Scherneck). SASH1 on chromosome region 6q24 is one of these recently identified candidate tumor suppressor genes (Zeller et al., Oncogene 22, 2972-2983, 2003). Some of the somatic alterations in these genes have been correlated with clinico-pathological observations.

die Funktion der Proteine, die bei höheren Eukaryoten an diesem Prozess beteiligt sind, allmählich klarer wird. Aspekte der Replikationsinitiierung werden derzeit sowohl an *Drosophila* als auch an Säugerzellen untersucht (M. Gossen).

Ein besonderes genetisches Element (genannt Sleeping Beauty-Transposon) weist eine hohe transpositionelle Aktivität in Zellen von Wirbeltieren auf (Z. Ivics). Daher ist dieses Transposon Element ein hilfreiches Werkzeug der Gentechnik zur Entdeckung von Genen in Wirbeltieren, sowie zur Analyse molekularer Mechanismen von Transposon-Wirt-Zellinteraktionen. Je nach Insertionsort werden induzierbare Transkriptionen von Promotoren innerhalb des Transposons eine Überexpression und/oder ektopische Expression endogener Gene oder Genteile bewirken. Dies kann zur Entdeckung neuer Onkogene und Tumorsuppressoren sowohl in Gewebekulturen als auch in lebenden Organismen genutzt werden. Da die Transposition die Generierung von DNA-Schäden mit sich bringt, kann das Sleeping Beauty-Transposon als experimentelles System zur Erforschung der grundlegenden molekularen Mechanismen dienen, die an der DNA-Reparatur beteiligt sind. Es konnte kürzlich nachgewiesen werden, dass sowohl nicht-homologe Endverbindungs- als auch homologe Rekombinations-Reparaturwege an der Reparatur durch Transposition-induzierter DNA-Schäden beteiligt sind (Izsvak et al., Mol. Cell 9, 147-156, 2004).

Strukturelle und funktionale Genomik

Die systematische Analyse der dreidimensionalen Strukturen von Proteinen, allgemein bekannt als strukturelle Genomik, wurde durch technische Neuentwicklungen in der Produktion rekombinanter Proteine ermöglicht (U. Heinemann). Ein Konsortium von Forschungsinstitutionen aus dem Raum Berlin-Brandenburg hat entsprechende Einrichtungen und eine technische Methodik geschaffen, die die Strukturbestimmung menschlicher Proteine mittels NMR-Spektroskopie und Röntgen-Kristallographie in wesentlich kürzerer Zeit und mit erheblich größerer Präzision ermöglicht als bisher (Heinemann et al., Acc. Chem. Res. 36, 157-163, 2003). Zu den bislang aufgeschlüsselten menschlichen Proteinstrukturen zählen die des Translationsinhibitors p14.5 (ein Protein, das in Leber- und Nierentumoren herunterreguliert wird), Gankyrin (das Produkt eines mit Leberzellenkarzinomen assoziierten Gens, das mit dem Retinoblastom-Protein interagiert), die Zyklin-abhängigen Proteinkinase 4 und 6, das 26S-Proteasom und Bet3p, eine zentrale Untereinheit des membranbindenden TRAPP-Komplexes, der an der cis-Golgi-Membran angesiedelt ist.

Computer-Simulation der Struktur von Makromolekülen bietet Einblick in deren Konformationsübergänge und Dynamik, die von Methoden der experimentellen Strukturbestimmung nicht geliefert werden können (H. Sklenar). Ein neuer Simulationsalgorithmus (Monte-Carlo), der die Vorhersage sequenzabhängiger DNA-Strukturen und die Untersuchung der Interaktionen von DNA-Liganden ermöglicht, ist etabliert und getestet worden. Zinkfinger-Proteine wurden in einer genomweiten Computer-Suche bei *Drosophila melanogaster* und *Arabidopsis thaliana* identifiziert (S. Böhm). Eine neue

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 cells show a constitutive activity of transcription factors such as AP-1 and NF- κ B. The latter signaling pathway appears to be partly responsible for the apoptosis resistance of HRS cells by the upregulation 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 appears 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* (Jundt et al. Blood 99, 3398-3403, 2002). Remarkably, ligand-induced Notch signaling was also identified as a critical growth factor for cultured and primary multiple myeloma cells suggesting that these interaction in the context of the bone marrow micro-environment contribute to myelomagenesis *in vivo* (Jundt et al., Blood, published online Jan. 15, 2004).

Little is known about the mechanisms of tumor rejection and the intricate interaction of T cells, antigen presenting cells, and tumor cells during this process. Inflammatory cytokines, such as IL-4 and IFN- γ , are important factors influencing tumor immunity (T. Blankenstein). For example, IFN- γ mediated angiostasis has been shown to be a general mechanism and critical requirement for tumor rejection by CD8⁺ T cells (Qin et al. Cancer Res. 63, 4095-4100, 2003). In addition, a novel mechanism has been discovered by which IL-4 contributes to tumor rejection by acting on tumor associated stromal cells (Schüler et al., J. Exp. Med. 198, 1487-1493, 2003).

Autoreactive T cells that have escaped the control mechanisms of the immune system may cause severe autoimmune diseases. In addition, genetic and poorly defined environmental factors also have an influence on the induction of these diseases (K. Falk/O. Röttschke). Small molecular compounds containing H-bond donor groups have been shown to drastically accelerate the exchange of peptide antigens presented by MHC class II molecules on the surface of activated antigen presenting cells (Falk et al., J. Biol. Chem. 277, 2709-2715, 2002). This mechanism bears the risk of loading activated antigen presenting cells with peptides derived from autoantigens and, consequently, results in the activation of autoreactive T cells.

A critical aspect in cancer immunotherapy is the generation of a strong, tumor-specific immune response. Retrovirally transduced genetically modified T cells expressing T cell receptors specific for tumor-associated antigens may help to facilitate adoptive therapy (W. Uckert). As T cell-mediated immune responses depend on the efficient presentation of antigen by dendritic cells, it might also be possible to enhance the therapeutic efficacy of vaccination strategies by modulating the proliferation, differentiation, or function of dendritic cells (A. Pezzutto).

Dendritic cells are derived from hematopoietic progenitors but the early steps in lineage decision and dendritic cell matu-

Zinkfinger-assoziierte Domäne (ZAD) wurde identifiziert (Chung et al., EMBO Rep. 3, 1158-1162, 2002).

Brust- oder Ovarialkarzinome treten normalerweise sporadisch auf. Ein gewisser Anteil (~ 10%) der Brustkrebsfälle ist jedoch erblich bedingt und wird durch Mutationen in den Tumorsuppressorgenen BRCA1 oder BRCA2 verursacht. Weitere Gene von hoher und geringer Penetranz, die mit Brustkrebs in Verbindung stehen, werden gegenwärtig durch eine Kopplungsanalyse in Familien mit hohem Risiko und in Assoziationsstudien an großen Patientengruppen identifiziert (S. Scherneck). Das Gen SASH1 in der Chromosomregion 6q24 ist einer dieser kürzlich identifizierten Tumorsuppressorgen-Kandidaten (Zeller et al., Oncogene 22, 2972-2983, 2003).

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 NF- κ B und AP-1. Insbesondere NF- κ B 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 (Jundt et al. Blood 99, 3398-3403, 2002). Das Signaling über Notch ist darüber hinaus ein wichtiger Faktor für das Wachstum primärer und kultivierter Plasmazytom-Zellen und ist daher vermutlich auch im Mikromilieu des Knochenmarks an der Entstehung von Plasmazytomen beteiligt (Jundt et al., Blood, published online Jan. 15, 2004).

Die molekularen Mechanismen der Tumorabstoßung, insbesondere die komplexe Interaktion von T-Zellen, Antigen-präsentierenden Zellen und Tumorzellen ist bislang wenig charakterisiert. Inflammatorische Zytokine wie z.B. IL-4 und IFN- γ sind dabei für die Tumormunität von besonderer Bedeutung (T. Blankenstein). Bei der IFN- γ -vermittelten Angiostase handelt es sich um einen generellen Mechanismus und eine wichtige Voraussetzung für die effiziente Tumorabstoßung durch CD8⁺ T-Zellen (Qin et al. Cancer Res. 63, 4095-4100, 2003). Darüber hinaus wurde ein neuer Mechanismus entdeckt, über den IL-4 über die Tumor-assoziierten Stromazellen zur Tumorabstoßung beiträgt (Schüler et al., J. Exp. Med. 198, 1487-1493, 2003).

Autoreaktive T-Zellen, die den Kontrollmechanismen des Immunsystems entkommen, können schwere Autoimmunerkrankungen hervorrufen. Zusätzlich sind aber auch genetische Faktoren und Umwelteinflüsse, wie kleine Moleküle, die bisher wenig charakterisiert sind, für den Ausbruch dieser Krankheiten mit verantwortlich (K. Falk/O. Röttschke). Kleine Moleküle mit funktionellen Gruppen, die als Wasser-

ration remained largely unknown (M. Zenke). By performing large-scale gene expression analysis, the inhibitory helix-loop-helix (HLH) transcription factor Id2 has now been identified as a major regulator of DC differentiation (Hacker et al. *Nat. Immunol.* 4, 380-386, 2003). Mice lacking expression of Id2 also lack several subsets of dendritic cells and, in addition, the balanced expression of Id2 and an activating HLH factor, such as E2A, is probably important for the differentiation of the hematopoietic progenitor cells into either B cells or dendritic cells.

Immune system homeostasis and adaptive immunity require that naïve lymphocytes constantly recirculate through secondary lymphoid organs. Lymphocyte and dendritic cell entry into the T and B cell zones of secondary lymphoid organs is largely regulated by the chemokine receptors CXCR5 and CCR7. Moreover, it has been shown that the balance of responsiveness to chemokine ligands for CXCR5 and CCR7, which are made in separate but adjacent zones, mediates B cell relocation in response to antigen (Reif et al., *Nature* 416, 94-99, 2002). Mice deficient for the chemokine receptor CXCR5 lack several lymph nodes and Peyer's patches. Although lymphoid organ development is unaffected in CCR7-deficient mice, it turned out that both chemokine receptors cooperate in lymph node development as mice double-deficient for CXCR5 and CCR7 lack all but mesenteric lymph nodes (Müller et al., *Immunol. Rev.* 195, 117-135, 2003).

stoffbrücken-Donor wirken, beschleunigen den Austausch von Peptiden, die durch MHC-Klasse II Moleküle auf der Oberfläche von aktivierten Antigen-präsentierenden Zellen präsentiert werden (Falk et al., *J. Biol. Chem.* 277, 2709-2715, 2002). Dieser Mechanismus birgt jedoch die Gefahr, dass aktivierte Antigen-präsentierende Zellen mit Autoantigenen beladen werden und auf diesem Weg autoreaktive T-Zellen aktivieren.

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, so dass ihre T-Zell-Rezeptoren spezifisch ein Tumor-assoziiertes Antigen erkennen, sind für eine adoptive T-Zell-Therapie besonders geeignet (W. Uckert). Da eine T-Zell vermittelte Immunantwort von einer effektiven Präsentation des Antigens durch dendritische Zellen abhängt, kann die Effizienz von Vakzinierungsstrategien über die Modulation der Proliferation, Differenzierung oder Funktion dendritischer Zellen verbessert werden (A. Pezzutto).

Über die Differenzierung dendritischer Zellen aus hämatopoetischen Vorläuferzellen ist noch wenig bekannt (M. Zenke). Mit Hilfe einer Microarray-basierten Analyse der Genexpression dendritischer Zellen wurde Id2, ein inhibitorischer Helix-loop-helix (HLH) Transkriptionsfaktor, als ein zentraler Faktor für die Differenzierung dendritischer Zellen identifiziert (Hacker et al. *Nat. Immunol.* 4, 380-386, 2003). Anscheinend ist die Balance der Expression von Id2 und eines aktivierenden HLH Proteins, wie z. B. E2A, für die Richtung der Differenzierung der Vorläuferzellen in B-Zellen oder dendritische Zellen von Bedeutung.

Die physiologische Migration von Lymphozyten sowie die Einwanderung von Lymphozyten und dendritischen Zellen in die T- und B-Zell Zonen sekundärer lymphatischer Organe wird maßgeblich durch die Chemokinrezeptoren CXCR5 und CCR7 gesteuert. Darüber hinaus sind diese Rezeptoren auch für die präzise Lokalisierung von B-Zellen in den Mikrokompartimenten sekundärer lymphatischer Organe verantwortlich, indem die Responsivität gegenüber den Liganden von CXCR5 und CCR7 moduliert wird (Reif et al., *Nature* 416, 94-99, 2002). CXCR5-Knock-out-Mäusen fehlen verschiedene periphere Lymphknoten, während die Entwicklung von Lymphknoten in CCR7-Knock-out-Mäusen kaum betroffen ist. Überraschenderweise zeigte sich bei der Analyse von Doppel-Knock-out-Mäusen für CXCR5 und CCR7, dass beide Rezeptoren in der Entwicklung von Lymphknoten kooperieren, da diese Mäuse bis auf die mesenterialen keine weiteren Lymphknoten ausbilden (Müller et al., *Immunol. Rev.* 195, 117-135, 2003).

Epithelial Signal Transduction, Invasion, and Metastasis

Walter Birchmeier

Our laboratory concentrates on the molecular analysis of epithelial morphogenesis and differentiation. In previous years, we defined the adhesion and signaling capacities of the E-cadherin/catenin/Wnt system. Moreover, we have investigated the role of scatter factor/hepatocyte growth factor (SF/HGF) and its receptor, the c-met tyrosine kinase, in morphogenesis of epithelial cells. Components of the Wnt and c-met pathways are mutated in a variety of human tumors.

Epithelial cells can lose expression of E-cadherin during tumor progression and this loss correlates with the appearance of highly invasive carcinoma cells. We have recently identified the new E3 ubiquitin ligase, Hakai, which results in E-cadherin degradation in a tyrosine-phosphorylation dependent manner (Fujita et al., 2002). The function of cadherins depends strictly on cytoplasmic linkage molecules, β -catenin, plakoglobin, p120, which mediate interaction of cadherins with the cytoskeleton. We have also shown that β -catenin binds to the transcription factor LEF-1/TCF and that this interaction translocates β -catenin to the cell nucleus and regulates gene expression (Behrens et al., 1996). This provides a molecular mechanism for the transmission of Wnt signals to the cell nucleus, which is essential in many developmental processes and in tumor progression (Huelsken et al., 2001; Soshnikova et al., 2003; Morkel et al., 2003). In the absence of Wnt signals, β -Catenin is degraded by the Axin/Conductin/GSK3 β /CK1 ϵ system (Behrens et al., 1998; Schwarz-Romond et al., 2002).

The scatter factor/c-met system transduces various signals in epithelial cells, such as scattering, differentiation, and proliferation. A unique activity of SF/HGF and c-met on epithelial cells in culture is the ability to induce branching or other morphogenic events. We have identified a new substrate of c-met, Gab1, which mediates the signal responsible for branching morphogenesis (Weidner et al., 1996; Schaeper et al., 2000; Sachs et al., 2000). Gab1 is a member of the family of membrane-bound multiadapter proteins, which transmits signaling of tyrosine kinase receptors (C. Birchmeier, et al., 2003).

Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-Cadherin complex

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In epithelial cells, tyrosine kinases induce tyrosine phosphorylation and ubiquitination of the E-cadherin complex, with results in endocytosis of E-cadherin. With a modified yeast 2-hybrid system, we isolated Hakai, an E-cadherin binding protein, which is an E3 ubiquitin-ligase. Hakai contains SH2, RING, zinc-finger and proline-rich domains and interacts with E-cadherin in a tyrosine phosphorylation-dependent manner, inducing ubiquitination of the E-cadherin complex. Expression of Hakai in epithelial cells disrupts cell-cell contacts and enhances endocytosis of E-cadherin and cell motility. Through dynamic recycling of E-cadherin, Hakai can thus modulate cell adhesion and could participate in the regulation of epithelial-mesenchymal transitions in development or metastasis.

Genetic interaction between Wnt/ β -catenin and BMP receptor signaling during formation of the AER and the dorsal-ventral axis in the limb

Natalia Soshnikova, Dietmar Zechner and Jörg Hülsken. In collaboration with Richard Behringer (Univ. Texas, Houston), Makoto Taketo (Univ. Kyoto), and Brian Crenshaw (Univ. Philadelphia).

By conditional gene ablation in mice, we found that β -catenin is a key regulator of the formation of the apical ectodermal ridge (AER) and of the dorsal-ventral axis of the limbs. By generation of compound mutants, we also show that β -catenin acts downstream of the BMP receptor IA in AER induction but upstream or parallel in dorsal-ventral patterning (see Figure). Thus, AER formation and dorsal-ventral patterning of limbs are tightly controlled by an intricate interplay between Wnt/ β -catenin and BMP receptor signaling.

The ankyrin repeat protein Diversin recruits Casein kinase I ϵ to the β -catenin degradation complex and acts in both canonical Wnt and Wnt/JNK signaling

Thomas Schwarz-Romond, Christian Asbrand, Hans-Jörg Schaeffer, and Jörg Hülsken. In collaboration with Jürgen Behrens (Univ. Erlangen), Matthias Hammerschmidt and Jeroen Bakkens (MPI Freiburg), and Michael Kühl (Univ. Ulm).

An alternative branch of the Wnt pathway uses JNK to establish planar cell polarity in *Drosophila* and gastrulation

We have analyzed the interactions between Wnt/ β -catenin and BMP receptor signalling during limb development using conditional mutagenesis, which allowed us to introduce loss-of-function (β -cat^{lox}) and gain-of-function ($\Delta N\beta$ -cat^{lox}) mutations of β -catenin, the central and essential mediator of canonical Wnt signalling. In addition, we generated compound mutant mice that carry both a gain-of-function mutation in β -catenin and loss-of-function mutations in Bmp receptor IA ($\Delta N\beta$ -cat^{lox}; BmpRIA^{lox}). Our analysis of these compound Bm4Cre; $\Delta N\beta$ -catenin^{lox}; BmpRIA^{lox/lox} mutant mice demonstrates that β -catenin acts downstream of the BMP receptor IA in the induction of the proximal-distal axis (AER, apical ectodermal ridge). In contrast, our data suggest that β -catenin acts upstream or in parallel to the BMP receptor IA during dorsal-ventral patterning. The intricate interactions between the Wnt/ β -catenin and BMP-signaling pathways provide the molecular basis that connects the development of proximal-distal and dorsal-ventral axes in the limb, and might thus ensure a tight spatial-temporal control of signalling responses.

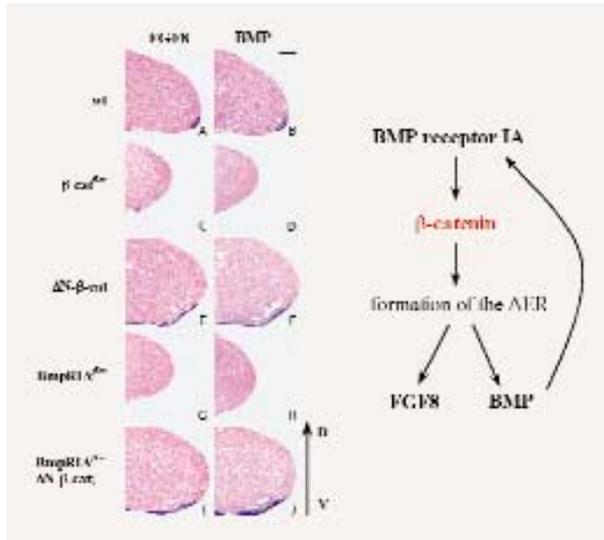


Figure A β -Catenin acts downstream of the BMP receptor IA during AER formation (see scheme on the right). (A-D) AER is absent or only few groups of cells resembling AER are present in β -catenin loss-of-function mutant limbs at the 42 somite stage. (E,F) AER and overall size of limb are strongly enlarged in β -catenin gain-of-function mutants at the 42 somite stage. (G,H) AER is not formed in Bmp receptor IA loss-of-function mutants at the 42 somite stage. (I,J) Limbs are strongly enlarged and the AER is expanded to ventral side in compound mutants at the 42 somite stage (cf. E,F). Dorsal-ventral is as indicated. Bar, 100 μ m.

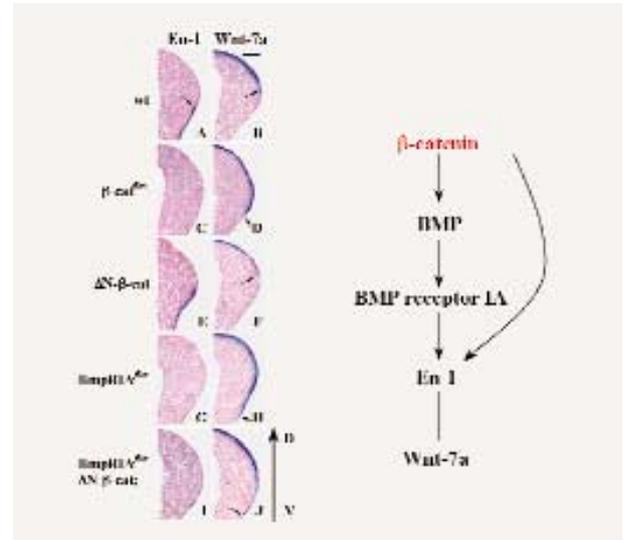


Figure B β -Catenin acts upstream of the BMP receptor IA during dorsal-ventral patterning of limbs (see scheme at right). (A,C) En-1 is not expressed in ventral limb ectoderm of mutants carrying loss-of-function mutation of β -catenin at the 30 somite stage. (B,D) Wnt-7a is expressed ectopically in ventral ectoderm of β -catenin loss-of-function mutant limbs at the 30-somite stage. (E,F) En-1 and Wnt-7a expression domains in β -catenin gain-of-function mutants resemble the wild type. (G) En-1 is not expressed in ventral ectoderm of mutants carrying loss-of-function mutations of Bmp receptor IA. (H) Wnt-7a is expressed in both dorsal and ventral ectoderm in mutants carrying loss-of-function mutation of Bmp receptor IA. (I) En-1 is not expressed in the ventral ectoderm of compound mutants at the 30 somite stage. (J) Wnt-7a is expressed in ventral limb ectoderm of compound mutants at the 30 somite stage (cf. H). Dorsal ventral is as indicated. Bar, 50 μ m.

movements in vertebrates. We have identified the novel vertebrate protein Diversin that interacts with two components of the canonical Wnt pathway, Casein kinase I ϵ (CKI ϵ) and Axin/Conductin. Diversin recruits CKI ϵ to the β -catenin degradation complex that consists of Axin/Conductin and GSK3 β and allows efficient phosphorylation of β -catenin, thereby inhibiting β -catenin/Tcf signals. Morpholino-based gene ablation in zebrafish shows that Diversin is crucial for axis formation, which depends on β -catenin signaling. Diversin is also involved in JNK activation and gastrulation movements in zebrafish. Diversin is distantly related to Diego of *Drosophila* that functions only in the pathway that controls planar cell polarity. Our data show that Diversin is an essential component of the Wnt-signaling pathway and acts as a molecular switch that suppresses Wnt signals mediated by the canonical β -catenin pathway and stimulates signaling via JNK.

β -Catenin regulates Cripto and Wnt3-dependent gene expression programs in mouse axis and mesoderm formation

Markus Morkel and Jörg Hülsken. In collaboration with Maki Wakamiya and Richard Behringer (Univ. Texas, Houston), Jixiang Ding and Michael Shen (UMDNJ Piscataway), Makoto Taketo (Univ. Kyoto) and Marc van de Wetering and Hans Clevers (Univ. Utrecht).

Gene expression profiling (Affimetrix) of β -catenin, Cripto, and Wnt3 mutant mouse embryos has been used to characterize the genetic networks that regulate early embryonic development. We have defined genes whose expression is regulated by β -catenin during formation of the antero-posterior axis and the mesoderm and have identified Cripto, which encodes a Nodal co-receptor, as a primary target of β -catenin signals both in embryogenesis as well as in colon carcinoma cell lines and tissues. We have also defined groups of genes that are regulated by Wnt3/ β -catenin signaling during primitive streak and mesoderm formation. Our data assign a key role to β -catenin upstream to two distinct gene expression programs during antero-posterior axis and mesoderm formation.

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Morkel, M., J. Huelsken, M. Wakamiya, J. Ding, M. van de Wetering, H. Clevers, M.M. Taketo, R.R. Behringer, M.M. Shen, and Birchmeier, W. (2003). Beta-catenin regulates Cripto- and Wnt3-dependent gene expression programs in mouse axis and mesoderm formation. *Development* 130, 6283-6294.

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Genetics of Tumor Progression and Metastasis

Ulrike Ziebold (Helmholtz Fellow)

Our laboratory concentrates on the unraveling of mechanisms underlying tumor formation, progression, and metastasis. It is well established that the change of a normal cell into a metastatic cancer cell arises due to the accumulation of mutations in proto-oncogenes and tumor suppressors. In our group, we would like to understand the consequences of these molecular defects. Therefore, we will rely first on established and newly created mouse cancer models and, secondly, on genetically modified murine embryonic stem cells (mES).

The nature of the interaction of pRB and E2F

The retinoblastoma protein (pRB), one of the first tumor suppressor proteins identified, is known to initiate tumors in both humans and mice. Recently, we have shown that E2F3, one member of the E2F-family, is a key downstream target of pRB. Surprisingly, E2F3-loss was able to promote or suppress the development of specific tumors. This demonstrated for the first time that, in the absence of Rb, E2F3 possesses tumor suppressive functions. Currently, we are analyzing this tumor suppressive function of E2F3 in the development of mammary tumors. We also established novel E2F3 inducible knock-in mice. Ultimately, we wish to uncover the full biological tumor functions of E2F3 and pRB.

Finding novel molecules that regulate progression of tumors and metastasis

In collaboration with M. Morkel, J. Fritzmann, W. Birchmeier and P. Schlag, at the MDC and the Robert-Rössle-Klinik/Charité.

Using *Rb/E2f3* mutant mice that develop aggressive mouse medullary thyroid carcinomas (MTCs), which metastasize to numerous organs, we hope to gain new insight into the nature of common and distinct regulators of the onset of metastasis. Currently, we are using "micro-array gene-chip" analysis of staged mouse and human tumors for comparative analysis. Potential candidates of our array will be tested in functional

migration and invasion assays. In the future, we would like to understand how these molecules regulate tumor progression and metastasis.

Mechanisms of tumorigenesis in murine embryonic stem cells

We will embark on aiding the detection of tumors by searching for novel marker genes and mechanisms for tumor initiation using murine embryonic stem cells (mES). Undifferentiated mES have the potential to grow tumors if transplanted into hosts. It is thus our hypothesis that the transition phase from the undifferentiated to the differentiated state is the focal point of tumor-suppressor and proto-oncogene action. We are developing a gene-trap screen to molecularly dissect the phenotypic changes of this transition phase by monitoring all transcriptional changes. Mutant mES-lines of our screen enable us to directly assess *in vivo* functions of promising candidates in the mouse. Ultimately, we wish to test the hypothesis if there are molecules and mechanisms that innately connect the control of stem cell proliferation and differentiation with the initiation and progression of tumors.

Selected Publications

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S100A4/metastasin expression depends on mutated or wild-type beta-catenin in knock-out strains

U. Stein, W. Walther, P.M. Schlag. In cooperation with E.D. Harris, S.D. Mertins, and R.H. Shoemaker, National Cancer Institute, Frederick, MD, and T. Waldman, Georgetown University, Washington, DC

Beta-catenin has been implicated as an oncoprotein in colon and other cancer types. The effects of individual mutations and their potential role in the malignant phenotype are difficult to assess. In order to isolate the effect of beta-catenin-mutations, gene knock-out technology was used to create isogenic strains of the HCT116 human tumor cell line which express only one wild-type or one mutant allele of this gene. We have coupled this to DNA array technology to identify those genes whose expression is dependent on beta-catenin genotype. cDNA array analysis of the human colon carcinoma cell line HCT116 in comparison to a derived knock-out strain expressing only wild-type beta-catenin indicated dramatic forty-fold down-regulation of expression of the gene S100A4 (metastasin), known to be associated with the metastatic phenotype. This effect was confirmed by quantitative real time RT-PCR and evaluation of protein levels, and by extended study of additional knock-out strains and a naturally nullsomic tumor cell line NCI-H28. In NCI-H28 clones stably transduced with the mutated beta-catenin, S100A4 mRNA levels were increased up to 70-fold, and in clones harboring the wild type beta-catenin exclusively, S100A4 expression was enhanced approximately 10-fold. In vitro migration was enhanced in clones stably transduced with the mutated beta-catenin. EMSA showed binding of both the beta-catenin/TCF-4 complex and of TCF-4 to the S100A4 promoter region in HCT116 cells, but only the binding of TCF-4 was detectable in NCI-H28 cells. Promoter activity of different deletion mutants of the S100A4 promoter was dependent on the presence of the TCF-4 consensus sequence. Comparison of the S100A4 status in the wild-type and mutant knock-outs with the parental heterozygous cell line HCT116 and with NCI-H28 cells suggests that mutant beta-catenin drives expression of S100A4 and does so in a dominant fashion when present in the heterozygous state.

A newly identified gene 7a5 is of prognostic value for metastasis of human colon carcinomas

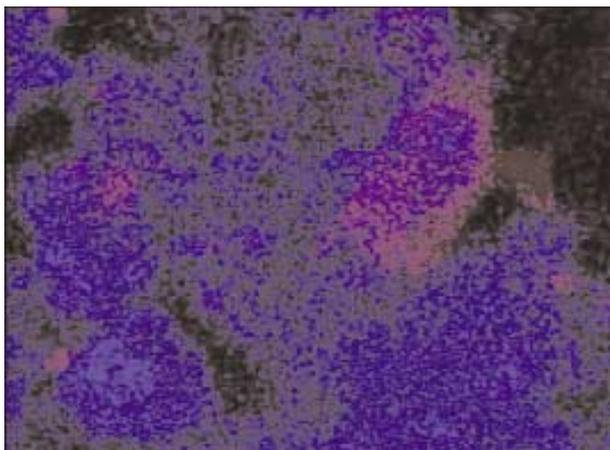
U. Stein, W. Walther, H. Schwabe, F. Arlt, P.M. Schlag. In cooperation with W. Birchmeier, MDC, I. Petersen, Institute of Pathology, Charité, I. Fichtner, MDC, and H. Kalthoff, University of Kiel

We identified a novel gene, preliminarily referred to as 7a5, by comparing the gene expression patterns in human primary tumors, metastases of different target organs, and in mucosa of colon cancer patients. We identified the full-length cDNA sequence of 2559 bp by using EST cluster analysis. The encoded putative protein of 852 amino acids harbors defined domains, such as a src-homology-domain (SH3)-binding motif for interaction with other proteins, and potential tyrosine phosphorylation sites. The gene 7a5 is localized on the human chromosome 7. Stably 7a5-transduced clones were generated and showed higher migration behavior with respect to their 7a5-overexpression. In order to investigate the impact of 7a5 on in vivo tumor growth, stably 7a5-transduced tumor cell clones were subcutaneously applied in nude mice. These experiments revealed an increased growth of the 7a5-overexpressing tumors. After orthotopic transplantation, highest tumors volumes and masses were again determined in those tumors showing the highest expression of 7a5. In human colon carcinomas and their metastases, 7a5 expression was detected by in situ-hybridization, real time RT-PCR as well as by immunohistochemistry using a newly generated polyclonal antibody. Furthermore, we examined microdissected surgical specimens of more than 70 patients with non-metastasizing and metastasizing primary tumors, their metastases of different target organs, as well as the corresponding normal tissues. 7a5 expression levels are significantly increased in the malignant tissues when compared to the corresponding normal tissues and were found to be higher in the metastases versus primary tumors. More importantly, 7a5 expression levels were measured to be significantly lower in those primary tumors which did not metastasize, neither syn- nor metachronously (time observed: up to 120 month), versus tumors which developed metastases within the next 120 months. These results suggest a prognostic role for 7a5 in primary colon tumors in order to predict the probability of developing distant metastases.

Hyperthermia and response-associated genes in human tumors

U. Stein, P. Hohenberger, W. Walther, P.M. Schlag. In cooperation with K. Jürchott, H. Lage and M. Dietel, Institute of Pathology, Charité, S. Bates, National Cancer Institute, Bethesda, MD and T. Litman, University of Copenhagen

Isolated, hyperthermic limb perfusion (ILP) with rhTNF- α (TNF) and melphalan is a highly effective treatment for advanced soft tissue sarcoma (STS) and locoregional metastatic malignant melanoma. Since multidrug resistance (MDR)-associated genes are known to be inducible by heat and drugs, expression levels of the MVP, MDR1, and MRP1 were determined sequentially before, during, and after ILP. STS or malignant melanoma patients were treated by ILP and tumor tissue temperatures were recorded continuously ranging from 33.4°C initially to peak values of 40.4°C during ILP.



Intracellular distribution of jet-injected naked rhodamine-labeled plasmid-DNA in xenotransplanted human colon carcinoma tissue. The confocal laser scanning microscopy shows the plasmid-DNA (red) in close association to the nuclei (blue) of tumor cells within the tissue 30 minutes after jet-injection.

We found in 83% of the patients, that the expression of MVP was induced during hyperthermic ILP at the mRNA as well as at the protein level. Elevated MVP protein expressions were observed either simultaneously with the MVP-mRNA induction, or timely delayed, following the induction at the transcriptional level. MVP-mRNA inductions often paralleled the increase in temperature during ILP. These temperatures and the drugs applied preferably led to an induction of MVP and were not sufficient to induce MDR1 and MRP1 in the majority of tumors. This study is the first to analyze the expression of MDR-associated genes sequentially during ILP of patients and demonstrates that treatment might lead to elevated levels of MVP, whereas enhanced levels of MDR1 and MRP1 remain rare events in the sarcomas and melanomas analyzed.

Moreover, a panel of thermoresistant and/or chemoresistant human gastric carcinoma sublines, which were continuously grown at elevated temperatures, was analyzed in order to evaluate the impact of ABC-transporters such as BCRP, MRP1, and MDR1 on thermoresistance. Expression of the ABC-transporters was found to be dependent on the classical or atypical type of chemoresistance. Furthermore, expression of the MDR-related ABC-transporters was increased in all thermoresistant counterparts, relative to the thermosensitive sublines, and overexpressed ABC-transporters were shown to be functionally active due to this long-term hyperthermic condition.

Expression profiling of early staged colon carcinomas

W. Kemmner, C. Astrosini, W. Haensch, P.M. Schlag. In cooperation with H.J. Gabius, Ludwig-Maximilians-University Munich, M. Höcker, Charité, A. Poustka, DKFZ, Heidelberg, and J. Reich, MDC, H. Okamoto, Tokohu University, Japan.

Survival of patients with early staged colorectal carcinomas showing neither lymph node nor distant metastases, is, in

most cases, more than five years. However, even in a group of patients with such a favorable prognosis, several of the patients die early due to post-operative formation of metastases. The aim of the present study was to identify genes which will allow detection of such high-risk patients. Expression profiles of colonic carcinomas were studied by oligonucleotide arrays using a novel strategy. Gene expression profiles of early staged colonic carcinomas from patients with a good survival were compared with those from patients with a poor prognosis. Since we suspect that normal mucosa of tumor patients is not as normal as expected, colonic mucosa of healthy individuals was taken as a reference point. In each case, colonic epithelium was captured using laser-microdissection. Processed and labeled RNA was hybridized to Affymetrix GeneChips U95A, U95B, U95C, U95D, and U95E containing about 60,000 sequences, most of them ESTs. No additional amplification of the RNA was performed, in order not to perturb the original representation of the RNA-sequences. Only sequences with a more than 4-fold differential expression difference were further examined. GeneChip analysis led to the identification of a number of candidate genes which show a strong deregulated expression between epithelial cells of healthy and tumor patients. Moreover, several genes showing a strong overexpression in the carcinoma cells of patients with poor survival could be identified. Evaluation of the candidate gene expression in about 60 cases of colorectal carcinomas with a well-documented follow-up 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 in some cases by immunohistochemistry. Meanwhile, 90 candidate genes arisen from the GeneChip experiments have been spotted onto glass slides. The feasibility of such a OncoChip comprising a definite set of putative marker genes for prognosis and diagnosis of colon cancers is now under examination.

Non-viral cancer gene therapy using jet-injection

W. Walther, U. Stein, R. Siegel, P.M. Schlag. In cooperation with EMS Medical GmbH, Nyon, Schweiz, and Plasmid Factory, Bielefeld

Various procedures are employed to deliver naked DNA into the desired cells or tissues *in vivo*. Among the various non-viral gene delivery technologies jet-injection is gaining increasing acceptance, since this technique allows efficient gene transfer into different tissues. In cooperation with EMS Medical a jet-injector prototype was created and tested for efficient *in vivo* gene transfer. The key parameters of *in vivo* jet-injection, such as jet-injection volume, pressure, jet-penetration into the tumor tissue, DNA stability, and bio-distribution of jet-injected naked DNA, have been analyzed for optimized non-viral gene therapy. Therapeutic *in vivo* experiments using the jet-injection transfer of the cytosine deaminase (CD) suicide gene demonstrated antitumor effects. In these *in vivo* studies, human colon carcinoma bearing NMRI-nu/nu mice were jet-injected with the CD gene harboring vector plasmid. Starting from day four of 5-fluorocytosine treatment, antitumor effects were seen in the CD-gene transduced tumors compared to the non-jet-injected control group. The growth inhibitory effect lasted for the entire observation time

of 24 days and showed significant growth inhibition of the jet-injected colon carcinomas compared to the non-transduced but 5-fluorouracil treated animals.

Based on our *in vivo* experiments, a phase I-study is planned to evaluate the feasibility of jet-injection aided LacZ reporter gene transfer in human colon- and mammary tumors. The results of this trial will provide the basis for the use of jet-injection to apply therapeutic genes for the treatment of patients with locally advanced colorectal cancer. These genes will be regulated by hyperthermia-inducible vectors to combine gene therapy with hyperthermia.

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Molecular Genetics of Cell Differentiation & Tumorigenesis

Achim Leutz

Introduction

Hematopoietic stem cells in the bone marrow continuously generate terminally differentiated blood cells of many different cell lineages such as erythrocytes, granulocytes, or macrophages. In addition, hematopoietic stem cells sustain their own maintenance - a condition called self-renewal. De-regulated cell differentiation or defective control of self-renewal may cause various diseases such as immune defects or leukemia. Thus, hematopoiesis provides striking examples to address fundamental biological and clinically relevant questions related to self-renewal of stem cells, cell lineage commitment, restricted progenitor proliferation, and cell differentiation control.

Combinatorial control of gene expression

Cell development and differentiation programs are accomplished by switching on and off distinct sets of genes. Gene regulatory proteins (transcription factors) that are downstream of signaling cascades bind to control regions of developmentally important genes and suppress or activate their expression. Specificity is primarily achieved by combinatorial control, i.e., through physical and functional interactions between several transcription factors that are simultaneously required at the same target genes. Combinatorial gene switches permit plasticity of regulation and allow a multitude of developmental decisions with a limited number of regulators. Many of such important gene regulatory proteins, however, are also prone to tumorigenic conversion by mutations.

Several years ago, we identified the first combinatorial molecular switch that instructs cells to express myeloid genes. The switch consists of two types of transcription factors that are also involved in leukemogenesis, namely: 1) proteins of the CCAAT-/Enhancer Binding Protein family (C/EBP) regulate differentiation and cell cycle arrest and 2) the product of the Myb proto-oncogene that is essential for the development and maintenance of all hematopoietic lineages. In a concerted

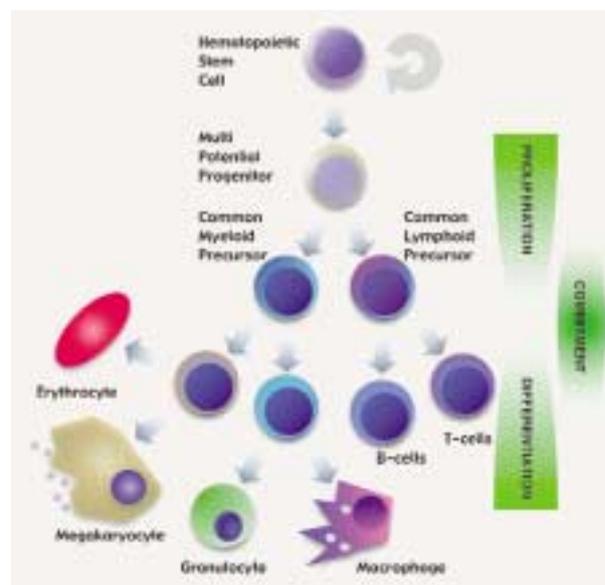
action, both transcription factors instruct myeloid gene expression, even in fibroblasts. Mutations in either transcription factor may abrogate their collaboration, disrupt the myeloid differentiation program, and contribute to leukemia. This concept has meanwhile been extended to several other co-operating hematopoietic transcription factors in different cell lineages.

Chromatin remodeling and lineage specific gene expression

A prerequisite for gene activation is to overcome the repressive effects of chromatin and to instruct polymerase II for transcription. C/EBP α and - β interact with the chromatin remodeling SWI-/SNF complex and with 'Mediator', a complex that bridges transcription factors with the basic transcription machinery. The interaction between SWI-/SNF and C/EBP is required to modify chromatin and to activate silent differentiation genes in concert with other transcription factors, such as Myb in the hematopoietic system or PPAR γ in adipogenesis. Moreover, interaction with SWI/SNF is also required for C/EBP α 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.

Schematic representation of hematopoiesis.

Hematopoietic stem cells in the bone marrow can either self-renew (gray 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.



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

C/EBP β is an intrinsically inhibited transcription factor that acts as a repressor in the absence of signaling and that is turned into an activator by the Ras oncoprotein. C/EBP β is phosphorylated through the Ras/MAP-kinase pathway and this phosphorylation event is accompanied by a conformational change of C/EBP β . We found that active and repressive C/EBP β 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. In its repressive form, C/EBP β preferentially binds to repressive Mediator whereas oncogenic Ras signaling selects the transcriptionally active Mediator complex that also associates with RNA polymerase II. This suggests that a Ras-induced structural alteration of C/EBP β determines differential gene activation through selective interaction with distinct Mediator complexes.

Translational regulation of transcription factors

From several hematopoietic transcription factor mRNAs, different protein isoforms arise by initiation of translation at alternative start sites. The resulting transcription factor isoforms harbor distinct N-termini domains that may organize different co-factor complexes with distinct functions in gene regulation. Hence, regulation of translation initiation may play a crucial role in the control of cell fate. We have shown that this is the case with C/EBP α , - β , and the Stem Cell and T-Cell Leukemia Transcription Factor, and Scl/Tal1, Distinct isoforms of these transcription factors display specific functions in proliferation control, activation of genes, and differentiation. These findings suggest an important role of translation initiation control in hematopoiesis and leukemogenesis.

Alternative initiation of translation may be prompted by small upstream open reading frames (uORF) that are located in the 5' region of the respective mRNAs. Such uORFs perceive the activity of the translation initiation machinery that is sensitive to environmental inputs such as stress, nutrition, or hormonal changes. This ancient mechanism of gene regulation is already found in yeast and allows cells to rapidly adjust to environmental changes. Proteins involved in translational control include PI3-kinase, AKT-kinase, PTEN-phosphatase or translation initiation factors eIF-2 α or eIF-4E, many of which can turn into oncogenes. It is anticipated that pathways and factors involved in the control of translational initiation may play a far more important role than previously recognized and that they represent novel targets for innovative drug therapies. Accordingly, we are developing screening systems to discover appropriate drugs.

Transcription co-factors

Transcription factors interact with other transregulatory proteins that function as co-activators, co-repressors, chromatin remodeling factors, and/or bridging factors between gene

regulatory complexes. These proteins provide “missing links” in understanding the complexity of gene regulation. We are therefore searching for proteins that interact with hematopoietic transcription factors and oncoproteins using the “yeast-two-hybrid system” or by protein purification affinity protocols. A number of interesting proteins have already been identified, e.g., proteins that harbor domains implicated in chromatin regulation during development or proteins with catalytic activity implied in the posttranslational modification of the transcription apparatus. We are developing murine knockouts and knock-ins as well as RNAi strategies to determine the effects of C/EBP mutants that are defective for interactions with several of these co-factors.

Selected Publications

Mo X., Kowenz-Leutz E., Xu H., and Leutz A. (2004) Ras Induces Mediator Complex Exchange on C/EBP β . *Molecular Cell*: 13:1-10

Calkhoven C. F., Müller C., Martin R., Krosch G., Pietsch H., Hoang T., and Leutz A. (2003) Translational control of SCL isoform expression in hematopoietic lineage choice. *Genes & Development*, 17:959-964

Pedersen T.A, Kowenz-Leutz E., Leutz A. and Nerlov C. (shared first and senior authorships) (2001) Cooperation between C/EBP α TBP/TFIIB and SWI/SNF recruiting domains is required for adipocyte differentiation. *Genes & Development*, 15:3208-3216

Calkhoven, C.F., Müller, C., and Leutz, A. (2000) Translational control of C/EBP alpha and C/EBP beta isoform expression. *Genes & Development*, 14: 1920-1932

Kowenz-Leutz, E. and Leutz, A. (1999) A C/EBP-beta Isoform Recruits the SWI/SNF Complex to Activate Myeloid Genes. *Molecular Cell*, 4: 735-743

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Translational Control of Gene Expression

Cornelis F. Calkhoven (Helmholtz Fellow)

Our work focuses on the expression of key-regulators in cellular differentiation, proliferation, and function at the mRNA-translation level. Research in the field of translation control has progressed rapidly, revealing new regulatory mechanisms and constantly augmenting the list of translationally regulated genes. Recently, the etiologies of several human diseases were linked to mutations in genes of the translational control machinery, highlighting the significance of this regulatory mechanism. In addition, deregulation of translational control is associated with a wide range of cancers. Novel therapeutic strategies are being developed that target translational control, a promising concept in the treatment of human diseases.

We study the translationally controlled expression of the transcription factors CCAAT/Enhancer Binding Protein (C/EBP) α and $-\beta$ and Stem Cell Leukemia factor (SCL) which are essential in differentiation programs of different systems including hematopoiesis. We have shown that various protein isoforms of C/EBP and SCL are expressed from alternative translation initiation sites. The isoforms have distinct and partially opposing functions in gene regulation, differentiation, and proliferation control. We showed, for the

first time in vertebrate cells, that translationally regulated expression of SCL and C/EBP protein isoforms determines cell fate. In the hematopoietic system, the ratio of SCL isoforms determines lineage outcome of primary bone marrow cells into either erythrocytes or megakaryocytes. We also demonstrated that translational deregulation of C/EBP isoform expression results in disturbed adipocyte differentiation and cellular transformation. Hence, translational deregulation of C/EBP isoform expression may be implicated in the development of tumors and metabolic disorders. Similarly, deregulation of SCL translation might be involved in its oncogenic potential as displayed in T-cell leukemia.

We discovered small upstream-open-reading-frames (uORFs) in the *c/ebp* and *scl* mRNAs that serve as *cis*-regulatory elements controlling the site of translation initiation. By monitoring the activity of translation initiation factors (eIFs), they determine the ratio of protein isoform expression. In this way, signal transduction pathways, which converge on ribosome activities, are linked to cell fate.

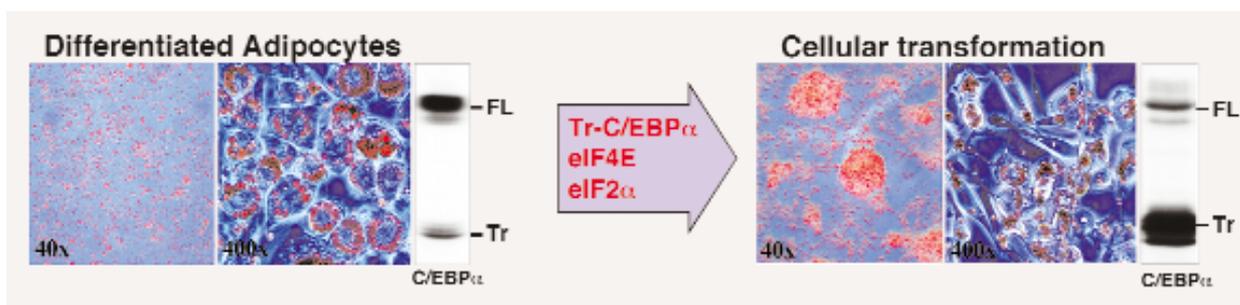
Several proteins of the translational control signaling network and machinery, as well as translationally controlled genes, are implicated in oncogenic, neurological, inflammatory, and metabolic disorders. It is anticipated that translational control in vertebrate development and disease will prove to be of greater importance than previously thought and that it may include targets for therapeutics. Therefore, we have created a Translational-Control-Reporter-System (TCRS) designed for the identification of such agents to aid in the development of novel therapeutic strategies in treating cancer and other human diseases.

Selected Publications

Calkhoven, C.F., Müller, C., Martin, R., Krosch, G., Pietsch, H., Hoang, T. and Leutz, A. (2003). Translational control of SCL isoform expression in hematopoietic lineage choice. *Genes & Dev.* 17, 959-964.

Calkhoven, C.F., Müller, C. and Leutz, A. (2002). Translational control of gene expression and disease. *Trends in Molecular Medicine.* 8, 577-583.

Upregulation of truncated (Tr) C/EBP α isoform expression results in cellular transformation of differentiated 3T3-L1 adipocytes. Aberrantly high levels of Tr-C/EBP α were caused by the upregulation of translation initiation factor eIF4E or eIF2 α activities, or ectopic overexpression of Tr-C/EBP α . 3T3-L1 cells underwent a differentiation protocol, were fixed and stained for lipid droplets with Oil Red O as described in Calkhoven et al. (2000). (Fl) full-length C/EBP α ; (Tr) truncated C/EBP α .



Calkhoven, C.F., Müller, C. and Leutz, A. (2000). Translational control of C/EBPa and C/EBPb isoform expression. *Genes & Dev.* 14, 1920-1932.

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Signal Transduction in Tumor Cells

Claus Scheidereit

The main interest of our laboratory is to understand how signal transduction processes are coupled to transcription. A model system with wide physiological and medical relevance is nuclear factor kappaB (NF- κ B) and its co-regulators and accessory proteins. A major goal of our research is to understand the structures and mechanisms underlying gene regulation by this complex system and its implications in disease development.

Pathways and structures that regulate NF- κ B activity

The pleiotropic transcription regulator NF- κ B plays a central role in the inducible expression of a large number of genes which encode cytokines, surface receptors, adhesion molecules, transcription factors, and other molecules controlling various immune functions as well as cell proliferation and programmed cell death. In its inactive latent form, NF- κ B is kept in the cytoplasm by association with I κ B molecules, which inhibit nuclear translocation and DNA binding activity of NF- κ B. Stimulation of cells with a variety of agents, such as bacterial lipopolysaccharides, tumor necrosis factor α , (TNF α) or interleukin-1 β (IL-1 β), results in the proteolysis of I κ B molecules and liberation of active NF- κ B into the nucleus. Induced I κ B proteolysis is triggered by I κ B phosphorylation mediated by an I κ B kinase (IKK) complex, which is activated by many NF- κ B stimulating pathways and consists of catalytic (IKK α , IKK β) and regulatory (IKK γ) components.

Differential regulation of NF- κ B activity by small I κ Bs and precursor molecules

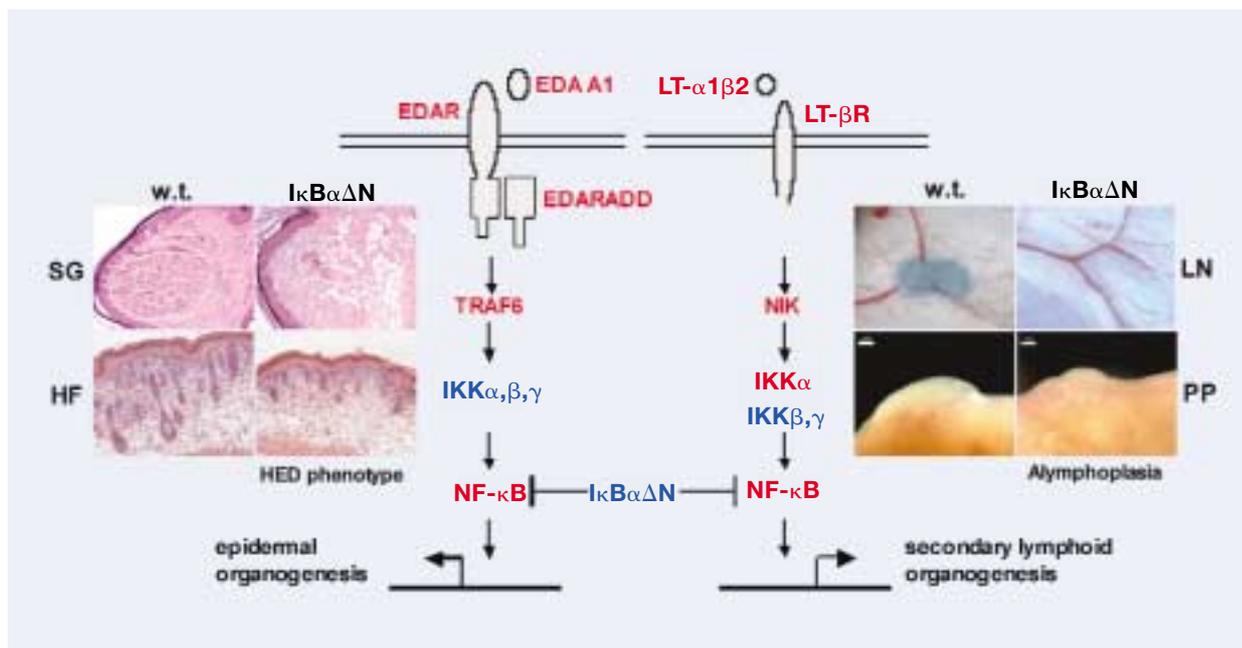
The mammalian NF- κ B family consists of five members, p50, p52, p65, c-Rel, and RelB. These proteins form hetero- and homodimers and are bound by the inhibitory cytoplasmic I κ B molecules I κ B α , β and ϵ , or by the nuclear I κ B homo-

logues Bcl-3 and MAIL. As an intriguing feature of NF- κ B transcription factors, two of its subunits, p50 and p52, are produced as precursor proteins, p105 and p100, respectively, which require proteolytic processing by the proteasome. Unprocessed p105 and p100 bind to other NF- κ B subunits and so act as cytoplasmic inhibitors.

The signal-activated IKK complex phosphorylates I κ B α , β , and ϵ at a conserved signal response domain and this sequence, containing also lysines for phosphorylation-dependent ubiquitin-conjugation, is sufficient to confer inducible degradation of the I κ Bs. In addition to this "classical" activation pathway triggered by IKK-mediated destruction of small I κ Bs, IKK controls the fate of the NF- κ B precursors as well. On stimulation with pro-inflammatory NF- κ B activating agents, such as tumor necrosis factor (TNF α) or bacterial lipopolysaccharides (LPS), cellular p105 is phosphorylated by IKKs at two serine residues. To bind the IKK complex, p105 contains an IKK docking site located in a death domain, which is separate from the substrate site. Upon phosphorylation by IKK, p105 attracts the SCF E3 ubiquitin ligase substrate recognition molecule β TrCP, resulting in polyubiquitination and complete degradation by the proteasome. p105-associated NF- κ B subunits, such as its own processing product p50, which is continuously formed by basal processing of p105, are then liberated and are transported to the nucleus to affect gene expression. In contrast to the signal-induced complete degradation of p105, the structurally related p100 molecule undergoes stimulus-dependent processing. We have shown that lymphotoxin and LPS, but not other activators of classical NF- κ B, such as TNF α or IL-1, induce proteolytic maturation of cytoplasmic p100 to its p52 product, which then migrates to the nucleus and binds to DNA. Most surprisingly, the processing reaction, which involves IKK-induced p100 polyubiquitination and partial digestion by the proteasome, can only take place while p100 is being synthesized at the ribosome, not after translation is completed.

Requirement of NF- κ B for embryonic development of hair follicles, eccrine glands, and formation of secondary lymphoid organs

Gene knockout studies in mice have revealed that single NF- κ B and IKK subunits are essential for various steps in immune responses and inflammation, but also for bone-morphogenesis and keratinocyte differentiation. However, due to embryonic lethality and functional redundancy between subunits, other physiological functions of the NF- κ B system were inaccessible. With a conditional gene targeting approach, we have ubiquitously expressed an NF- κ B super-repressor, I κ B α Δ N, to broadly downmodulate NF- κ B activity in the entire organism or in single organs and tissues. Mice with suppressed NF- κ B display macrophage dysfunction and the lack of secondary lymphoid organs like Peyer's patches and peripheral lymph nodes. NF- κ B inhibition further causes severe defects in the early developmental steps of epidermal appendages, including hair follicles and tear and sweat glands. Normally, these structures display strong NF- κ B transcriptional activity, as we have demonstrated with β -galactosidase reporter mice. The epidermal phenotype is analogous to



Two major signaling pathways essential for organogenesis, which require NF- κ B activity (Schmidt-Ullrich, 2001, *Development* 128, 3843). A knock-in strategy, where the NF- κ B superrepressor I κ B Δ N was expressed in mice, revealed that NF- κ B is required to confer Ectodysplasin signals for the embryonal development of hair follicles (HF), sweat glands (SG) and other epidermal structures, the lack of which establish the hypohidrotic ectodermal dysplasia (HED) phenotype (left). NF- κ B is also required for transmitting lymphotoxin signals to induce formation of secondary lymphoid organs (peripheral lymph nodes, LN, and Peyer's patches, PP) (right). Signaling molecules which upon inactivation cause similar defects in both pathways are indicated in red.

hypohidrotic (anhidrotic) ectodermal dysplasia (HED) in humans, and identical to phenotypes of *eda*, *edar* or *crinkled* mice. The *eda* and *edar* genes belong to the TNF family of ligands and receptors, respectively. Our data thus indicate that NF- κ B is required in epidermal development for *edar* to transmit *eda* signals. Currently, the crosstalk of NF- κ B with other signaling pathways during epidermal organogenesis is being investigated.

Role of IKK/NF- κ B and AP-1 signaling cascades in Hodgkin's disease

In collaboration with the research group of Bernd Dörken, we previously discovered an essential role of aberrant constitutive NF- κ B activity in the viability of Hodgkin's disease (HD) tumor cells. The NF- κ B/I κ B system is dysregulated in a cell-autonomous manner, generally involving a persistent activation of the IKK complex. Constitutive NF- κ B blocks apoptosis and promotes proliferation and tumorigenicity of the malignant cells. We have now undertaken a genome wide determination of genes which are activated by constitutive IKK/NF- κ B in Hodgkin's disease. NF- κ B accounts for the high level expression of a large group of genes, many of which represent characteristic expression markers for the malignant cells. Thus, NF- κ B drives expression of genes which regulate cell cycle progression, inhibit programmed cell death, direct tropism, and migration of tumor cells, their resistance to chemotherapeutic drugs and abundant cytokine production, indicating a central pathogenetic role of the IKK/NF- κ B pathway.

We have found that in addition to IKK/NF- κ B, malignant cells of Hodgkin's disease bear a second aberrantly activated transcription factor. AP-1 (activating protein 1), composed of the c-Jun and JunB subunits, is strongly overexpressed. c-Jun and JunB overexpression is observed in the great majority of tumor cells of all patients tested with classical Hodgkin's disease, to a lesser extent in anaplastic large cell lymphoma, but not in other B or T cell malignancies. Further analysis revealed that AP-1 supports proliferation of Hodgkin cells. In fact, AP-1 cooperates with NF- κ B to co-stimulate expression of the cell-cycle regulator cyclin D2 and the lymphocyte homing receptor CCR7, which are all strongly expressed in Hodgkin cells. These data suggest an important role for AP-1/c-Jun and for a cooperation between IKK/NF- κ B and AP-1 in Hodgkin lymphoma pathogenesis.

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Tegethoff, S., Behlke, J., and Scheidereit, C. (2003) Tetrameric oligomerization of IKK γ is obligatory for I κ B kinase complex activity and NF- κ B activation. *Mol. Cell Biol.* 23, 2029-2041.

Choi, M., Rolle, S., Wellner, M., Cardoso, C., Scheidereit, C., Luft, F.C. and Kettritz, R. (2003) Inhibition of NF- κ B by a

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Antigen Receptor Signaling in Lymphocytes

Daniel Krappmann (Helmholtz Fellow)

Antigen receptors on T or B lymphocytes are responsible for inducing opposite responses of immunity or tolerance. A number of positive and negative regulatory mechanisms have been described. In T cells, immunogenic activation requires TCR (T cell receptor) engagement by antigenic peptides and a second co-stimulatory signal, of which CD28 is the most prominent. Optimal TCR/CD28 ligation initiates a series of signal transduction events which modulate the activity of several nuclear transcription factors including NF- κ B, AP-1, and NF-AT, ultimately leading to differentiation and proliferation. In contrast, weak T cell activation, e.g. by poorly cross-linking peptides or the absence of co-stimulus, promotes an uncoupling of the TCR and their downstream signaling pathways and induces T cell tolerance. Paradoxically, the same antigen receptor triggers both outcomes. Resolving how one receptor elicits such opposing effects is crucial for determining how specific immune responses can be selectively switched on or off to counteract organ rejection or to treat autoimmune diseases.

Antigenic stimulation of the I κ B kinase (IKK) complex and subsequent activation of NF- κ B is essential for clonal expansion of naïve B and T cells. Using Jurkat T cells and gene target disruption in mice, novel PKC θ was identified as a central component coupling TCR proximal signaling events to IKK/NF- κ B activation. By the use of pharmacological inhibitors as well as rescue experiments in PKC θ deficient cells, we could demonstrate that also in mouse pre B cell lines and in primary B cells PKCs are essential for mediating B cell receptor (BCR) induced NF- κ B activation. In contrast, NF- κ B activation by cytokines or pathogenic stimuli is independent of PKCs. This study provided clear evidence that conserved upstream pathways trigger IKK activation in response to BCR and TCR clustering in both lymphocyte populations. This conservation was confirmed by more recent studies in which Bcl10, MALT1, and CARMA1 were identified as common signaling components required for bridging PKCs to IKK activation in response to antigen receptor stimulation in T and B cells.

In an approach to gain mechanistic insights into the processes that govern activation of the IKK complex after T and B cell activation, we have investigated post-translational modifications of the critical regulator Bcl10. PKC or T cell receptor (TCR)/CD28 signaling results in phosphorylation and subsequent down-regulation of Bcl10 protein levels, thereby attenuating NF- κ B transcriptional activity. Bcl10 degradation requires an intact caspase recruitment domain and is not observed after stimulation with TNF- α or LPS. Bcl10 down-regulation is not affected by proteasome inhibitors but is accompanied by transient localization to lysosomal vesicles, suggesting involvement of the lysosomal pathway rather than the proteasome. The HECT-domain ubiquitin ligases NEDD4 and Itch promote ubiquitination and degradation of Bcl10, thus down-modulating NF- κ B activation. Since CD3/CD28 induced activation of JNK is not affected by the decline of Bcl10, degradation of Bcl10 selectively terminates IKK/NF- κ B signaling in response to TCR stimulation. Together, these results suggest a novel mechanism of negative signaling in which TCR/PKC signaling initially activates Bcl10 but later promotes its degradation- a process that might help to prevent abnormal lymphocyte activation and induce tolerance.

Selected Publications

Scharschmidt, E., Wegener, E., Heissmeyer, V., Rao, A. and Krappmann, D. (2004) Degradation of Bcl10 induced by T cell activation negatively regulates NF- κ B signaling. *Mol. Cell. Biol.* (in press).

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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.

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 multi-spanning 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 mobilizes the ubiquitin-conjugated and dislocated molecules which are still attached to the cytosolic surface of 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 years. 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.

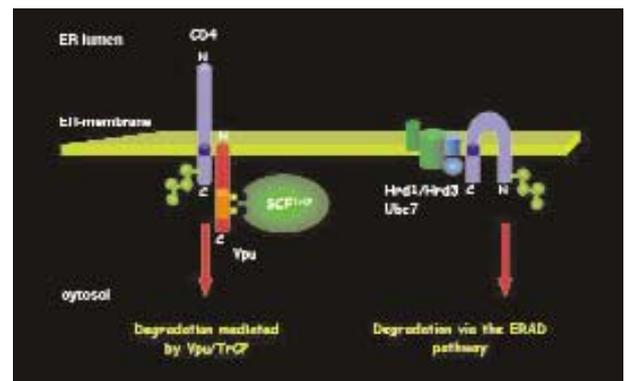
One example of this is the human immunodeficiency virus (HIV) induced degradation of the plasma membrane protein

CD4 during its maturation in the ER. This proteolysis is mediated by Vpu, an HIV-encoded N-terminally anchored membrane protein that interacts with CD4. In addition, Vpu binds the human F-box protein β TrCP, which functions as a substrate recognition factor of the multisubunit ubiquitin ligase SCF. β TrCP recognizes a specific degradation signal contained in the cytoplasmic tail of Vpu. This signal is also found in other short-lived substrates of the human SCF $^{\beta$ TrCP, like β -catenin and I κ B α , and comprises two phosphorylated serine residues. However, in contrast to β -catenin and I κ B α , binding of β TrCP does not lead to proteolysis of Vpu but instead to degradation of the associated CD4. However, ubiquitination of CD4 has not been shown. An additional open question is whether the known membrane-bound components of the ubiquitin system also participate in this process or whether Vpu mediated turnover of CD4 represents a separate pathway that may also be mechanistically distinct from ERAD of endogenous substrates.

To address these open questions, we have reconstituted Vpu mediated turnover of CD4 in yeast. A reconstitution in this model organism offers the advantage that mutants in all ERAD components can be used. Reconstitution of the viral degradation mechanism for CD4 was possible by co-expression of only Vpu and human β TrCP. Interestingly, this system is active in the absence of the membrane-bound ERAD components. Thus, the viral system seems to work independently of the endogenous ERAD pathway. In addition, we found that CD4 expressed in the absence of Vpu and β TrCP is a target of endogenous yeast ERAD. This unique situation allows a direct comparison of the viral and the cellular degradation pathways for the same molecule. Our detailed analysis of the proteolysis of CD4 revealed mechanistic differences between the viral and the cellular pathway. Taken together, our results point to the fact that HIV hijacks an unrelated proteolytic pathway, recruits it to the ER-surface, and uses it in degradation of CD4 from the membrane.

Figure 1
The two pathways for degradation of the integral membrane protein CD4 are outlined in this cartoon.

The viral mechanism involves the HIV encoded Vpu protein that binds to CD4. In addition, Vpu recruits the cytosolic ubiquitin ligase SCF $^{\beta$ TrCP to the ER-membrane, which attaches a polyubiquitin chain onto CD4. Degradation by the endogenous cellular ERAD system involves the classical ERAD components Hrd1p/Hrd3p and Ubc7p. Therefore, the viral mechanism is distinct from the cellular ERAD system. Furthermore, the viral mechanism depends on the presence of cytosolic lysine residues in the substrate as acceptor sites for ubiquitin. In contrast, the endogenous ERAD system does not depend on such cytosolic attachment sites, since it can transfer the lysine containing luminal parts of CD4 into the cytoplasm. Thus, both pathways differ mechanistically: The viral system cannot transport parts of CD4 without prior ubiquitination, while the cellular ERAD system is able to transport before ubiquitination takes place.



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Regulation of Nuclear Transport Processes

Katrin Stade (Helmholtz Fellow)

In eukaryotic cells, genetic information is stored in the nucleus, whereas protein synthesis occurs in the cytoplasm. This spatial separation of essential cellular processes, such as transcription and translation, can only be overcome by allowing macromolecules to traverse the nuclear membrane in both directions. Nucleocytoplasmic trafficking is therefore a major cellular activity, in terms of both number of individual particles involved and energy consumption. However, despite their enormous complexity, nuclear transport processes still offer an excellent opportunity for efficient regulation of cell cycle progression and signalling pathways. Our lab investigates a family of soluble nuclear transport receptors termed karyopherins which can be further classified as import and export receptors, so-called importins and exportins, respectively. Xpo1 was the first exportin to be described (1) and since then, detailed information has become available with respect to this particular transport receptor and the export pathway it is serving (2). However, much remains to be learned about Xpo1.

One of our current research interests is to identify Xpo1-specific export substrates and study their interaction with this exportin in more detail. In addition to nuclear protein export, a new research interest of the lab is the study of nuclear import processes and how they might be regulated by post-translational protein modification. In the past, protein phosphorylation was identified as an important means of regulating nuclear transport reactions. More recently, a rather 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 cell cycle progression, chromatin structure, and DNA repair, was also recognized as a key player for one particular nuclear protein import pathway in budding yeast (3). Ongoing studies in our lab now focus on the elucidation of SUMO's exact role for nuclear transport reactions.

Selected Publications

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Initiation 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 that contribute to the initiation of replication is poorly analysed. 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)- specifically, members of the minichromosome maintenance (MCM) protein family and of the Origin Recognition Complex (ORC). Currently, both *Drosophila* and mammalian tissue cultures are used as experimental systems. In addition, the group is interested in transcriptional cross-talk between neighboring transgenes and the long-term stability of their expression patterns.

Localization of the *Drosophila* Origin Recognition Complex (ORC)

ORC is likely to function as the initiator protein in eukaryotes (i. e. its binding to chromosomal sites specifies the origins of bidirectional 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, an embryonic development which relies on maternally supplied stockpiles of replication factors, and the finding that certain specialized origins in follicle cells can be monitored individually by confocal microscopic techniques. 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, and reveals changes in the dynamic behavior of this protein complex in different tissues and throughout development (see figure).

Biochemical characterization of the human ORC

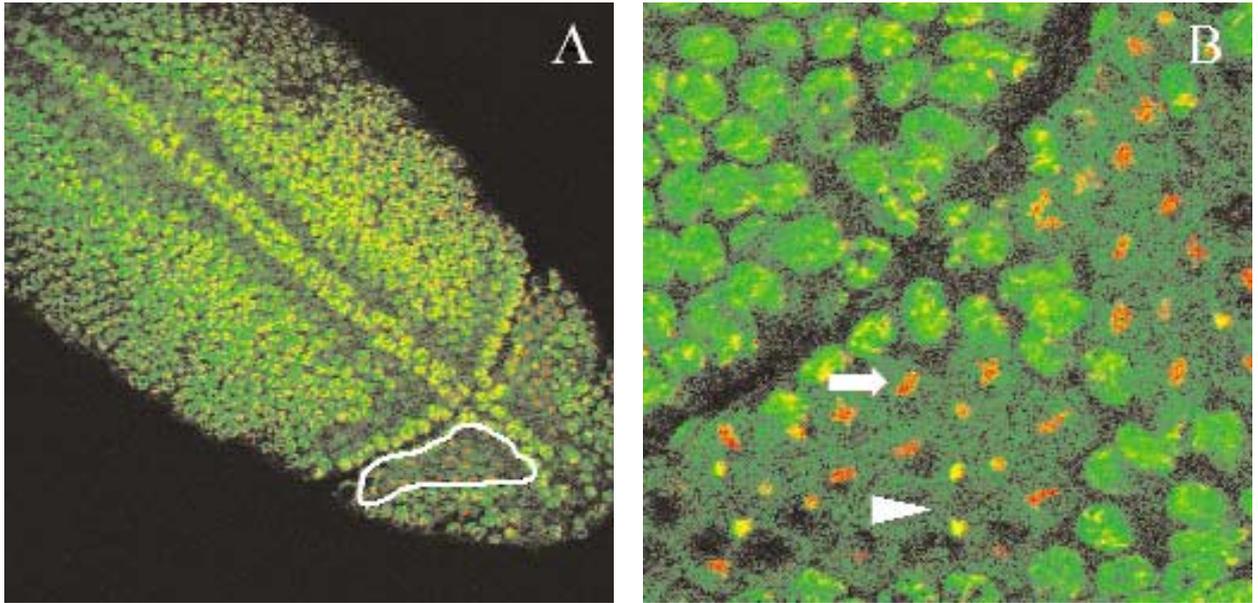
We were able to co-express genes for all six subunits of human ORC in insect cells and to purify the resulting protein complex to homogeneity. It turns out that human ORC is capable of forming various distinct sub-complexes, which differ in their stability and DNA binding properties. By omitting individual subunits in this protocol, specific interactions among the ORC proteins could be revealed. According to the *Saccharomyces cerevisiae* paradigm, ORC's binding to DNA is expected to be ATP-dependent. We are currently investigating this possibility by analyzing the subunits responsible for ATP binding as determined by nucleotide crosslinking and testing the biochemical properties of recombinant human ORC defective in ATP interactions. We will evaluate the significance of these findings for the role of ORC in DNA replication initiation by using an *in vitro* DNA replication protocol.

Ablation of MCM proteins

The genomes of all eukaryotic organisms code for six different MCM proteins which can interact with each other. Three of them, MCM 4, 6, and 7, form the putative replicative DNA helicase. The other subunits are believed to suppress or regulate the helicase activity. In a collaboration with atugen AG, Berlin, we are investigating the effects of special antisense molecules ("Geneblobs") directed against one of the MCM genes, Mcm4, in primary human fibroblasts. We have discovered that the knockdown of this gene results in the growth arrest of the cells, the stop of DNA synthesis, and an increase in the G2/M population of the transfected cells. Interestingly, some other members of the MCM protein family are also efficiently down-regulated. The mechanisms involved in this process are currently under investigation.

Interference of closely spaced transcription units

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 analysing the effects these transgenes exert on each other by using an inducible transcription system. Upon induction of a target gene, a neighboring, "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 analysed taking the effects of epigenetic transgene control like DNA methylation and chromatin compaction into account.



Drosophila ORC2 is excluded from metaphase chromosomes.

A) Confocal microscopy of a 3 to 4 hour old rescue embryo (Orc2-GFP; Orc2^{-/-}). The Orc2-GFP signal is shown in green, DNA counterstain in red (only merged pictures are shown). Encircled in white is one of the mitotic domains, i. e. a patch of adjacent cells still progressing in synchrony through the cell cycle at this stage of embryonal development. **B)** The same mitotic domain (depicted in a neighboring Z-plane) in higher resolution. Note that there is no enrichment of the GFP signal on metaphase chromosomes (white arrow), whereas in anaphase (white arrowhead) ORC2 is concentrated on the chromosome, with merged green and red channels resulting in the yellow signal.

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. In 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 participate in, or regulate, the transposition reaction, we identified the DNA-bending protein HMGB1 as a cofactor of SB transposition. HMGB1 most likely plays a role by assisting synaptic complex formation during transposition. Furthermore, we have shown that proteins involved in double-strand break repair are limiting factors of transposition in mammalian cells and that both homology-dependent and homology-independent repair pathways contribute to the success of a transposition event. 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 are currently concentrating our efforts on the following projects:

1. SB has a number of advantages as a gene vector when compared to current viral and non-viral gene transfer technologies. Our goal is to evaluate, develop, and modify the SB vector system so that it will become an efficient and safe vector for human gene therapy. Specifically, we are in the process of determining the rate at which transposon vectors integrate into chromosomes of non-dividing cells.

2. We propose to 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 transposase gene in the hope that we can derive hyperactive versions of the transposon system. With such hyperactive vectors, we hope to be able to efficiently knock out genes in vertebrate model organisms;
 - b) initiating 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 a transposon-based misexpression screen in mammalian cells in order to identify novel genes involved in tumorigenesis.

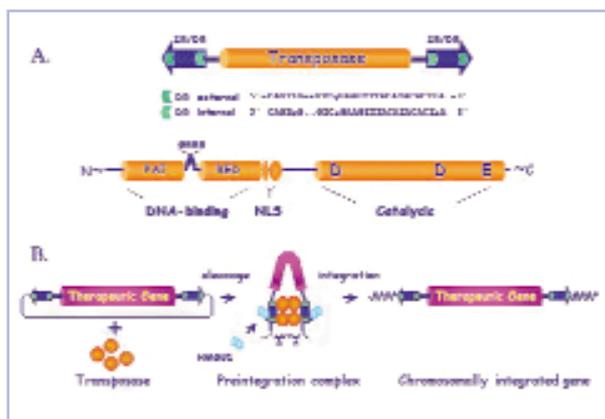
3. We are investigating the molecular interactions between transposons and host cells.
 - a) We are following a “candidate” approach by looking for interactions with cellular factors that are involved in other recombination systems. We established that high mobility group proteins, as well as proteins that are involved in double-strand DNA break repair, are host factors of transposition. We are in the process of investigating the involvement of other repair and/or cell cycle checkpoint proteins in transposition.
 - b) We also follow a “blind” approach by performing a yeast two-hybrid screen on a human gene library. With the screen, we have already identified two human proteins that specifically interact with the Sleeping Beauty transposase.
 - c) In search of 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

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

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.

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The Sleeping Beauty transposable element and its transposition.

(A) Components of the element. On top, a schematic drawing of the transposon is shown. The element has a single gene encoding the transposase, which is flanked by terminal inverted repeats (IR/DRs, blue arrows), each containing two binding sites for the transposase (small green arrows). A sequence alignment shows the actual sequences of the external and internal transposase binding sites. The transposase has an N-terminal, bipartite, paired-like DNA-binding domain containing a GRRR AT-hook motif, a nuclear localization signal (NLS) and the DDE catalytic domain. **(B)** Transposition. The transposase gene within the element can be replaced by a therapeutic gene, and the resultant transposon can be maintained in a simple plasmid vector. The transposase is supplied in trans. The transposase binds to its binding sites within the IR/DR repeats and, together with host factors such as HMGB1, forms a synaptic complex, in which the ends of the transposon are paired. The transposon is excised from the donor molecule and integrates into a new location.

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Structural Studies of Proteins and Nucleic Acids by X-ray Crystallography

Udo Heinemann

Biological processes in normal and diseased cells are best understood utilizing three-dimensional structures of the large molecules upon which they are based. Macromolecular crystallography is a uniquely powerful tool to study the structures of proteins, nucleic acids and their complexes, since it permits the determination of the precise arrangement of all atoms and the shape and property of all surfaces in single molecules as well as huge molecular complexes. This knowledge can be used to explain biochemical observations, to predict biological functions, and to design ligands specific to a site on the surface of given protein molecule. We combine X-ray diffraction studies with biochemical and biophysical studies of proteins involved in nucleic-acid binding or linked to disease states, in particular in the cancer field. Crucial to this work is the ability to prepare crystallizable samples of nucleic acids, proteins, and protein domains by chemical, biochemical, and gene-technological means. The underlying techniques have recently been adapted to medium-to-high throughput in the frame of the Protein Structure Factory, the first European structural genomics initiative.

Nucleic acid-interacting proteins

The homing endonuclease *PI-SceI* is an intein, an internal protein, embedded into the extein sequence of the vacuolar membrane H^+ -ATPase. In a protein splicing process, it releases itself from the precursor and combines the two exteins to a functional ATPase. Responsible for this activity is domain I of *PI-SceI*, which also contributes most of the energy required for specific binding to a DNA sequence of at least 31 bp length, the *VMA1-Δvde* locus on chromosome 8 of *Saccharomyces cerevisiae*. This locus is the allele of a *VMA1* gene (VMA: vacuolar membrane H^+ -ATPase) that is deficient of *vde* (VDE: VMA-derived endonuclease = *PI-SceI*). In a process called “homing”, domain II of *PI-SceI* cuts a specific recognition sequence at the *VMA1-Δvde* locus and initiates the insertion of the *vde* gene. *PI-SceI* thus has dual activities in catalyzing both protein and DNA splicing reactions. In collaboration with A. Pingoud (University of Gießen), we

have determined the crystal structure of domain I of *PI-SceI* at high resolution in order to clarify some open questions about the protein’s DNA-binding mode (Werner et al., 2002). The structure suggested a sub-domain motion in domain I to be required for site-specific DNA binding. This model was qualitatively supported subsequently by other research groups.

The process of partitioning ensures that dividing bacterial cells receive a full complement of the chromosomal or plasmid DNA. Low-copy-number plasmids often encode a ParA/ParB pair of proteins guiding this process, where ParA is an ATPase and ParB is a site-specific DNA-binding protein. In collaboration with E. Lanka (MPI for Molecular Genetics, Berlin), we are studying the structural basis of partitioning of the conjugative *Escherichia coli* plasmid RP4 whose proteins IncC and KorB serve as ParA and ParB homologs. As a first step towards a full structural characterization of the system, the crystal structure of the C-terminal domain of KorB (KorB-C) was determined (Delbrück et al., 2002). KorB-C was shown to be a closely linked dimer with subunits of SH3-like fold, and solution and mutagenesis data indicate that the C-terminal domain is indeed responsible for the dimerization of intact KorB. More recently, the structure of the central part of KorB, KorB-O, bound to a 17-bp DNA fragment containing the consensus KorB-binding sequence (O_B , present 12 times on RP4) was determined by X-ray diffraction methods. KorB-O was shown to be a completely α -helical protein that binds the DNA through major-groove contacts. Surprisingly, the specificity-determining contacts to the binding site are formed by amino-acid residues outside the helix-turn-helix motif of KorB. These findings were corroborated by mutagenesis studies.

Structural genomics of human proteins

Structural genomics is defined as a world-wide “large-scale project to determine the three-dimensional shapes of all proteins and other important biomolecules encoded by the genomes of key organisms”. The Max Delbrück Center is participating in this new approach to structural biology by assuming a leading role in the Berlin-area Protein Structure Factory (Heinemann et al., 2003). This consortium focuses on the systematic structure analysis of human proteins and develops high-throughput technology for expression cloning, protein crystallization, and synchrotron-based X-ray diffraction experiments. We have used the Protein Structure Factory approach to study a number of human proteins that are related to cancer and/or play important roles in cellular signaling or transport processes.



Structures of human proteins determined within the Protein Structure Factory. Clockwise from top left: Crystal structures of nicotinamide mononucleotide adenylyltransferase (NMNAT), gankyrin, a fumarylacetoacetate-family hydrolase member, hp14.5, the PX domain from p47^{phox}, Bet3p, and prolyl peptidase D. Center: NMR structure of the C-terminal BRCT domain from BRCA1 (left) and crystal structure of α -spectrin SH3 domain (right).

The human protein hp14.5 is a member of the large YjgF/YER057c/UK114 protein family which comprises more than 200 members. The gene encoding hp14.5 is down-regulated in kidney and liver tumors, whereas a high expression level is observed in fully differentiated cells. L-PSP, the rat homolog of hp14.5, is known to inhibit protein synthesis *in vitro*, probably due to its endoribonucleolytic activity towards single-stranded RNA. The crystal structure shows hp14.5 to be a symmetric trimer composed of chorismate-mutase-like subunits (Manjasetty et al., 2004a). Surface clefts at the subunit interfaces are lined with amino-acid residues conserved in the YjgF/YER057c/UK114 protein family. These clefts bind benzoate molecules from the crystallization buffer and are likely to function as active sites carrying a hydrolytic activity of the protein.

Gankyrin is a recently described oncoprotein linked to hepatocellular carcinoma. The protein is primarily composed of ankyrin repeats and interacts with a number of molecules, some of which are known to play important roles in cell signaling and cancer. Among these are the retinoblastoma protein (Rb), the cyclin-dependent protein kinases CDK4 and CDK6, the melanoma antigen (MAGE-A4), and the S6 ATPase of the 26S proteasome. Gankyrin counter-acts the kinase inhibitor p16^{INKA4}, also an ankyrin-repeat protein, and thereby modulates the CDK4-mediated Rb phosphorylation, reducing the stability of Rb. In the crystal, gankyrin is present as a monomeric protein with an extended surface formed by five complete and two terminal, incomplete ankyrin repeats

(Manjasetty et al., 2004b). A sequence motif, LXCXE (178LACDE182 in gankyrin), known to interact with Rb is in α -helical conformation, whereas it was found in extended, β -like structure in two different Rb-bound proteins. The crystal structure of gankyrin may serve as a starting point towards a full structural and biochemical study of gankyrin-ligand interactions.

In vesicle transport between cellular compartments, docking or tethering complexes residing on the target membrane mediate early steps of vesicle attachment that precede the pairing of SNARE proteins and membrane fusion. The TRAPP (*transport protein particle*) docking complex is involved in tethering of ER-derived COP-II vesicles to the *cis*-Golgi compartment. It consists of at least seven different polypeptides and can be immunoprecipitated with one labeled subunit, Bet3p. The crystal structure of Bet3p shows this protein to be dimeric with subunits of α/β -plait topology. Each subunit is covalently modified with a palmitate molecule covalently coupled to the sulfhydryl group of Cys68 *via* a thioester linkage. The palmitoyl moieties are deeply buried inside the protein, thus keeping it soluble in aqueous buffers. The structure suggests, however, a mechanism by which the fatty acid chains may be extruded from Bet3p into the Golgi membrane bilayer. It appears possible that the palmitoylation of Bet3p is involved in fixing the TRAPP complex to the target membrane. The Bet3p structure opens the exciting possibility to structurally characterize a complete tethering complex or sub-complexes thereof.

Selected Publications

Manjasetty, B.A., Delbrück, H., Pham, D.-T., Mueller, U., Fieber-Erdmann, M., Scheich, C., Sievert, V., Büssow, K., Niesen, F., Weihofen, W., Loll, B., Saenger, W. & Heinemann, U. (2004a) Crystal structure of Homo sapiens protein hp14.5. *Proteins: Struct. Bioinf.* 54, 797-800.

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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, 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.

In the last years, we have focussed 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 and their interactions with ligands and suggests that it too can be successfully used for simulations of protein structures and biological membranes.

Monte Carlo simulation algorithm

Daniel Wüstner

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 locally and allow for large conformational changes in Monte Carlo simulations. It is essentially this choice of independent variables that permits conformational equilibration on a reasonable computational time scale, which is a necessary condition for deriving meaningful structural data from the trajectories of molecular simulations. The algorithm has been implemented in several new programs for the simulation of nucleic acid structures, ligand-DNA complexes, and biological model membranes.

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 solved 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.

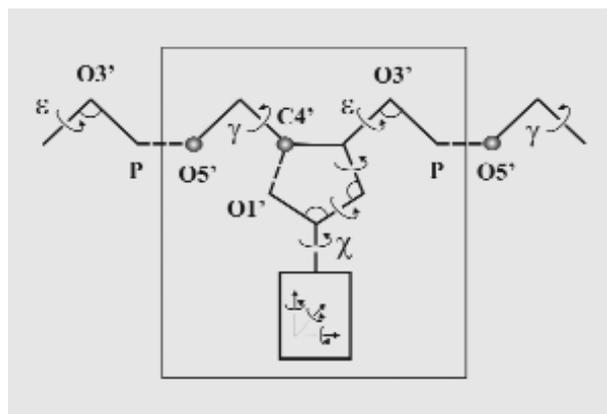
We have participated in an international initiative with the goal to improve the underlying data by using large-scale Molecular Dynamics simulations on the current state-of-the-art level. For this purpose, 39 DNA fragments, each 15 base pairs long and including all possible tetranucleotides, have been selected and simulated over 15 ns. Preliminary results, however, show that the simulation time is still too short for full conformational equilibration. It was therefore suggested that the simulations be repeated using our new Monte Carlo (MC) technique. Performance and results of this approach were demonstrated by applications to three DNA dodecamers with the palindromic sequences $d(CG)_6$, $d(TA)_6$, and $d(CGC-GAATTCGCG)$. 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 10^6 MC cycles, which need less than one week of 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.

DNA-ligand interactions

Remo Rohs

Methylene blue, an efficient singlet oxygen generating dye, binds to DNA and allows photosensitized reactions to be used for sequence-specific cleavage of the DNA backbone. Intercalation and groove binding are possible binding modes of the dye, depending on base sequences and environmental condi-

Currently implemented molecular model for Monte Carlo simulations of nucleic acid structures, including 14 independent chain and ring variables per nucleotide. The P-O5' and O1'-C4' bonds are chosen for chain breakage/closure, where the positions of atoms O5' and C4' are determined by the closure equations.



tions. According to experimental results, the dye intercalates into GpC and CpG base pair steps, but prefers minor groove binding in case of AT alternating DNA sequences. We have therefore extended our former modeling study of methyl-ene blue binding to a DNA decamer with AT alternating base sequence, in order to compare both sequences. The results show that our modeling technique faithfully reproduces the experimental data and, moreover, has enabled us to explain the significantly different binding behavior in energetic and structural terms. Although the relative stability of the different complexes is similar for the two sequences, subtle differences are seen in energy decompositions and can be attributed to the change from symmetric 5'-YpR-3' intercalation to minor groove binding with increasing salt concentration, which is experimentally observed for the AT sequence at lower salt concentration than for the GC sequence. This difference is due to a significantly lower non-electrostatic energy for the minor groove complex with AT alternating DNA, whereas the slightly lower binding energy to this sequence is caused by a higher deformation energy of DNA (including solvent contributions). The energetic data are in agreement with the conclusions derived from different spectroscopic studies and can also be structurally interpreted on the basis of the modeled complexes.

Conservation, diversification, and expansion of C2H2 zinc finger proteins in eukaryotic genomes

Siegfried Böhm

C2H2 zinc finger proteins (ZFPs) constitute the most abundant family of nucleic acid binding proteins in eukaryotes. The basic functions of C2H2 ZFPs range from DNA or RNA binding to their involvement in protein-protein interactions. In addition, a comparison of the whole ZFP sets in the main eukaryotic lineages has revealed a further level of ZFP complexity through their remarkable lineage specific diversification and expansion.

In the period reported, we have analyzed the full ZFP sets in the *Drosophila* and *Arabidopsis* genomes (dZFPs and aZFPs), with the aim of their classification in functional terms. This work was done in close collaboration with the groups of H. Jäckle (MPI Göttingen) and W. Mewes (IFB München). In the *Drosophila* genome we have identified 326 putative ZFP genes corresponding to ~2.3% of all annotated genes. 94 of the dZFPs are conserved in other animals. In addition, another ~1/3 of unique dZFPs contain a novel N-terminal zinc-finger-associated domain (ZAD), which is restricted to insects. ZAD-ZFPs constitute the most expanded subfamily of dZFPs and are clustered at few chromosomal sites. These features are reminiscent of the vertebrate specific KRAB-ZFPs. Based on the very recently solved crystal structure of the ZAD domain, it was shown that ZAD domains are involved in protein-protein interactions like the KRAB domains, suggesting their possible repressor function.

In the *Arabidopsis* genome we have identified 175 putative ZFP genes corresponding to ~0.7% of all annotated genes. Only 31 of the aZFPs are conserved in other eukaryotes. Their vast majority (144 out of 175) is unique to *Arabidopsis*. They are largely conserved in other plants and can therefore be con-

sidered as plant-specific ZFPs. Most of the unique aZFPs are derived from extensive duplication events in the *Arabidopsis* genome, resulting in several expanded ZFP families. The two largest families, named C1 and A1, are constituted by 64 and 24 members, respectively, and are suggested to be involved in transcriptional regulation.

The largest lineage specific ZFP families in *Drosophila* and *Arabidopsis* described above are completely unrelated and reflect the diversity of transcriptional regulation guided by ZFPs in animals and plants. In contrast, the few conserved ZFPs, found in animals and plants, should be involved in more ancient molecular processes like RNA metabolism and chromatin remodeling.

Selected Publications

Rohs, R. and Sklenar, H. (2004) Methylene Blue binding to DNA with alternating AT base sequence: Minor groove binding is favored over intercalation. *J. Biomol. Struct. Dyn.* 21, 699-711.

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Nucleosides, Nucleotides, and Oligonucleotides

Eckart Matthes

A new nucleoside-marker for tumor imaging by PET

[¹⁸F]-Fluorodeoxyglucose ([¹⁸F]FDG) is currently the most widely used radiotracer for imaging malignant tumors by positron emission tomography (PET). However, its application in cancer diagnosis, staging, and therapy monitoring is limited by the fact that it is more an indicator of glucose metabolism than of tumor proliferation. The development of more specific cancer imaging agents is currently the focus of intensive research.

In a common project with the Clinic for Nuclear Medicine, Charité – University Medicine Berlin (D. L. Munz), we have designed, synthesized, and investigated a series of modified thymidine analogs which meet biological and chemical requirements we consider essential for successful tumor imaging. Principally, such nucleoside analogs should be resistant against the nucleoside degrading enzyme thymidine phosphorylase and should be phosphorylated well by thymidine kinase 1, an enzyme whose activity is increased 10-20 fold in proliferating cells. The product of this enzyme reaction is a nucleoside monophosphate which is trapped inside of proliferating cells and can be detected by PET provided that the applied thymidine analog is labeled appropriately, e.g. with [¹⁸F].

We have found one promising new nucleoside which meets these requirements (FAT) and for which we have established a rapid and simple synthesis and a carrier-free nucleophilic [¹⁸F]- labeling and purification procedure. All these methods were adapted successfully to an automated PET tracer synthesizer (Nuclear Interface).

These results enable us to apply the new PET precursor to the detection of tumors in mice. Whole-body PET scans taken from mice with a subcutaneously grown malignant melanoma (B16) 1 h after i. v. application of 20 MBq [¹⁸F]-FAT have shown a highly significant uptake of the [¹⁸F] activity in the tumor compared with non-proliferating as well as with pro-

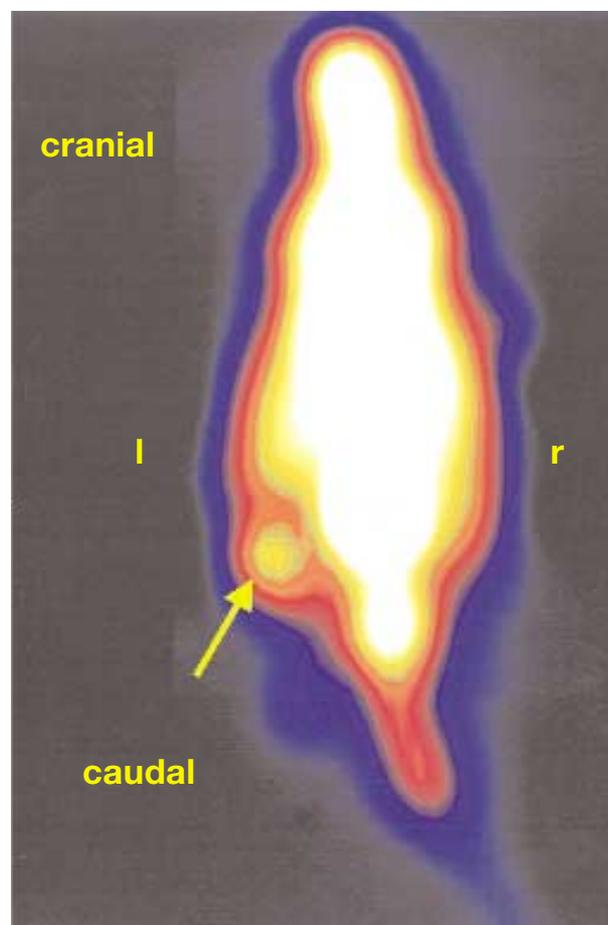
liferating tissues (Fig. 1). This and a second promising [¹⁸F]-PET precursor will be pursued with the aim to get an approval for clinical application.

Inhibitors of HBV and HCV replication

Recently, L-nucleosides, the stereoisomeric analogs of the naturally occurring D-nucleosides have gained tremendous interest as highly selective inhibitors of viral infections. In line with this development, we have focussed our interest on hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. In cooperation with H. Will (Heinrich-Pette Institute, Hamburg) and R. Bartenschlager (Molecular Virology, Otto-Meyerhof Center, University Heidelberg), we have designed and synthesized new L-deoxyribonucleoside and L-ribonucleosides targeting HBV DNA polymerase and RNA-dependent RNA polymerase (NS5B), respectively. Some highly selective inhibitors of HBV replication in HepG2 2.2.15 cells were detected and presented in a patent application.

To date, no L-ribonucleosides are known with antiviral activity against HCV. Surprisingly, we identified two compounds which inhibit HCV-replication in a Huh-7 cell-based replicon assay. They display only minimal effects on cellular RNA synthesis and cellular proliferation. Thus, further

Figure. Whole-body PET scan taken 1 h after i.v. application of 20 MBq [¹⁸F]-FAT. The coronal slice demonstrates the [¹⁸F]-FAT accumulation in the tumor located in the left flank (arrow).



chemical modifications are directed toward increasing their antiviral activity.

Telomerase detection by an optical biosensor

We have found earlier that phosphorothioate modified primers bind strongly to the primer binding site, a specific protein motif of telomerase resulting in a dramatic increase of the velocity of the enzyme reaction in comparison to unmodified primers.

In cooperation with the Fraunhofer Institute for Biomedical Techniques, Potsdam (F. Bier), we put these findings to practical use for the development of a new fiberoptical biosensoric system to detect telomerase activity in human tumors in real time without PCR.

For this purpose, FITC-labeled probes which were complementary to the newly synthesized telomeric DNA were added. For detecting signals, an evanescent-wave assay configuration was selected to take advantage of the total internal reflection fluorescence. Unpurified extracts of 10⁶ HL-60 tumor cells were found to be sufficient for a selective telomerase proof. Thus, our aim is to make this method more sensitive.

Selected Publications

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Tumor Genetics

Siegfried Scherneck

The research program of this group aims to better understand the genetic basis of cancer, particularly human breast cancers (BC). BC, one of the most common cancers affecting women, has been demonstrated to be a complex genetic disease with high clinical heterogeneity and characterized by the accumulation of multiple molecular alterations. At present, our knowledge about BC associated genes, their functions, and interactions in pathways regulating growth and arrest of BC cells is still limited.

Although about 90% of BC are sporadic, the remaining cases are heritable and caused by mutations of at least two tumor suppressor genes, BRCA1 and BRCA2. It is likely that more highly penetrant susceptibility genes will emerge. However, attention is now shifting toward more common low-penetrance mutations and their possible contribution to BC. Therefore, multiple approaches, such as linkage in high-risk families and association studies in large BC case-control studies, are being used to identify additional high- and low-penetrance genes.

Furthermore, new technological developments offer powerful means to analyze the activity of thousands of genes in samples of sporadic and hereditary BC at the DNA, RNA, and protein levels. Such analyses have proven to be valuable in identifying genes and pathways associated with different types of BC, response to treatment, and prognosis.

Comprehensive analysis of German breast/ovarian cancer families for BRCA1/BRCA2 germline mutations: Mutation profiles and frequencies in the German population

W. Hofmann, E. Claßen, D. Horn, B. Jandrig, S. Seitz, A. Nothnagel, I. Sümnick, H. Zeidler, K. Krause, A. Pietschmann, M. Hinzmann, the "Berlin Center for Hereditary Breast and Ovarian Cancer" in cooperation with the "German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC)"

Germline mutations in the BC susceptibility genes, BRCA1/BRCA2, jointly explain the most significant part of the familial breast/ovarian cancer syndrome. Within the GCHBOC, which was initiated and supported by the "Deutsche Krebs-hilfe", we have participated in a comprehensive study to analyze the entire coding sequence of the BRCA1 and BRCA2 genes in about 3,000 patients from German breast/ovarian cancer families. A total of about 100 BRCA1 and about 80 BRCA2 distinct deleterious mutations have been identified by the GCHBOC. More than one third of these mutations are novel and might be specific for the German population. The mutation study has defined groups of high-risk families. Mutation rates of only 50% and lower in the higher-risk groups provide evidence for further predisposing genes. At present, identified mutations and unclassified variants in the BRCA genes, as well as specific rearrangements in mutation carriers, have been characterized for clarification of genotype-phenotype correlations. In addition, the prevalence and penetrance of mutations of other genes for hereditary breast cancer, like CHEK2, have been analyzed.

Systematic search for genes contributing to the genesis and progression of sporadic and hereditary breast cancer

S. Seitz, B. Jandrig, M. Plaumann, A. Schwartz, C. Zeller, K. Wenzel, P. Waßmuth, D. Siele, J. Strissel, R. Frege, K. Poppe, S. Werner, in cooperation with A. Meindl (München), N. Arnold (Kiel), D. Niederacher (Düsseldorf), K. Schmutzler (Köln), P.M. Schlag, (RRK, Berlin), I. Petersen (Berlin), B. Hinzmann, A. Rosenthal (metaGen, Berlin), W. Arnold (atugen, Berlin).

Over the last few years, we have focused our research on genes whose function is impaired or lost during BC development on chromosome regions 6q24 (i.e. SASH1), 8p12-p21 (i.e. TRAIL-receptor genes, EXTL3) and 17p13.3 (i.e. PFN1). Some of the somatic genetic alterations (genes) identified have been correlated with clinico-pathological parameters.

To identify and validate BC associated genes, several positional and functional approaches are being used in combination: identification of differentially expressed ESTs by electronic and real Northern blotting and RT-PCR; fine mapping of LOH hotspots; microarray-technology; and mutation analysis. In addition, transfection assays and functional complementation tests have been applied.

Recently, we have developed a promising strategy to search for BC relevant genes by combining array based expression profiling of BC with a powerful functional approach; namely, the microcell mediated chromosome transfer in BC cells. We identified a common set of candidate genes which can be placed in different pathways. Several of the genes and pathways identified may prove to be useful in diagnosis and provide new targets for therapies directed against a BC tumor type.

Tumor suppressor activity of the microfilament protein profilin 1 in breast cancer

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

Profilin 1 (PFN1) is a regulator of the microfilament system and involved in various signaling pathways. Immunohistochemical analysis revealed intermediate and low levels of profilin 1 in different human BCs.

The importance of PFN1 for human tissue differentiation has been demonstrated by our research findings that human BC cells expressing low PFN1 levels adopt a non-tumorigenic phenotype upon raising their profilin-1 level. In a study to characterize the ligand binding sites crucial for PFN1 tumor suppressor activity in BC, we found that the actin binding site is instrumental for this activity.

Selected Publications

Zeller, C., Hinzmann, B., Seitz, S., Prokoph, H., Burkhard-Goettges, E., Fischer, J., Jandrig, B., Estevéz-Schwarz, L., Rosenthal, A., and Scherneck, S. (2003) SASH1: a candidate tumor suppressor gene on chromosome 6q24.3 is downregulated in breast cancer. *Oncogene* 22, 2972-2983.

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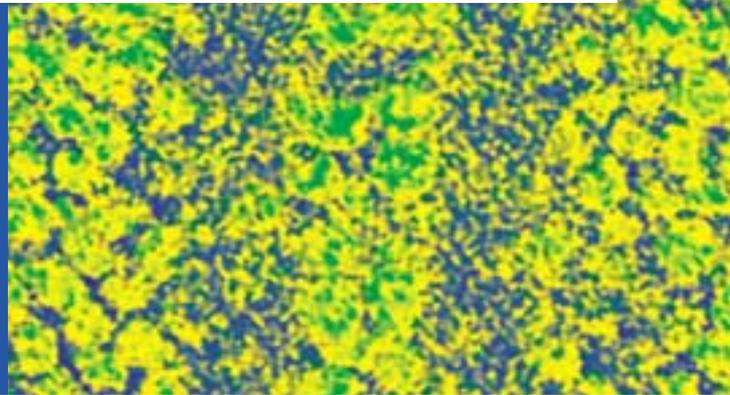
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Differentiation and Growth Control in Lymphocyte Development and Immunopathogenesis

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 in lymphoid organ development, systemic immune responses, and chronic inflammatory diseases. In addition, we are interested in the immune modulatory and growth-inducing functions of chemokine receptors encoded by human herpesviruses, and the function of sphingophospholipid receptors in the immune system.

Development and organization of lymphoid tissues by homeostatic chemokine receptors

Lymphocyte homing to lymphoid and nonlymphoid tissues, as well as lymphocyte recirculation between secondary lymphoid organs, critically depends on the chemokine system. According to their expression pattern and function in immune system homeostasis and immune responses, chemokines are broadly divided into two groups - homeostatic and inflammatory chemokines. Although there exists some redundancy in the chemokine system, chemokine receptor expression and receptor sensitivity on lymphocytes, as well ligand expression in lymphoid and peripheral tissues, appears to be tightly regulated. Expression of the chemokine receptors CCR7 and CXCR5 enables T cells, B cells, and dendritic cells to enter secondary lymphoid organs. In addition, they are responsible for the proper positioning of these cells within distinct microenvironments of lymphoid organs. For example, CCL19 and CCL21, ligands for CCR7, which are expressed by dendritic cells and stromal cells within the T cell zones of secondary lymphoid organs, retain T cells within this microenvironment. In contrast, the ligand for CXCR5, CXCL13, is expressed by follicular dendritic cells and stromal cells within B cell follicles and attracts B cells and subsets of T cells into

the B cell areas of lymphoid organs. Chemokines are also responsible for fine-tuning the localization of lymphocytes within microcompartments. B cell positioning at the boundary of B cell follicle and T cell zone in the spleen, where naïve, mature B cells interact with T cells recently activated in the adjacent T cell zone, has been shown to be controlled by the balanced responsiveness of B cells towards the ligands for CCR7 and CXCR5.

In addition to their function in the organization of lymphoid organ microarchitecture, CXCR5 and CCR7 are crucial for lymphoid organogenesis. Mice deficient for the chemokine receptor CXCR5 or its ligand CXCL13 lack several types of peripheral lymph nodes and the majority of Peyer's patches. In addition, both strains of mice show severe alterations in the morphology of the spleen and the remaining Peyer's patches. In contrast, secondary lymphoid organ development is largely unaffected in mice lacking the expression of the chemokine receptor CCR7 or in mice lacking the expression of its ligands within secondary lymphoid organs. However, by generating mice with a targeted deletion for both chemokine receptors, we have shown that CCR7 cooperates with CXCR5 in lymphoid organ development.

The expression of CCR7 and CXCR5 defines functionally distinct T cell subsets

CCR7 is the dominant chemokine receptor mediating T cell entry and positioning within secondary lymphoid organs. Its expression is tightly regulated during T cell differentiation from naïve towards memory/effector cells: Expression of CCR7 and CD62L, which are necessary for homing to secondary lymphoid tissues and highly expressed on naïve CD4⁺ T cells, are downregulated on terminally differentiated effector cells. Instead, effector cells express chemokine receptors for inflammatory chemokines that allow them to enter nonlymphoid tissues at sites of infection and inflammation. 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 that preferentially home to nonlymphoid organs. 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 (T_{CM}) cells. A third population, about 15% of human peripheral blood CD4⁺ T cells, expresses CXCR5 along with CCR7 and was provisionally named T_{CM1}. 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 CD154 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.

CCR7 controls cytotoxic T cell priming in alloimmune responses

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

The requirement of CCR7, which regulates 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. Additional prolongation of graft survival was observed when hearts from CCR7^{-/-} mice were used as donor organs. Our results define a key role for CCR7 in allogeneic T cell priming within the context of draining lymph nodes.

Immune modulatory and growth-inducing functions of viral chemokine receptors

We have previously shown that Epstein-Barr-Virus (EBV) specifically transactivates expression of the cellular chemokine receptor CCR7 by its regulatory nuclear factor EBNA2. In contrast to EBV, several other human herpesviruses, like cytomegalovirus (CMV) or the lymphotropic human herpesvirus type 6 (HHV-6) and Kaposi's sarcoma-associated herpesvirus (KSHV), also termed HHV-8, encode viral chemokine receptors and chemokines in their genomes suggesting that herpesviruses use the chemokine system to interfere with the growth and differentiation program of the host and subvert specific immune responses.

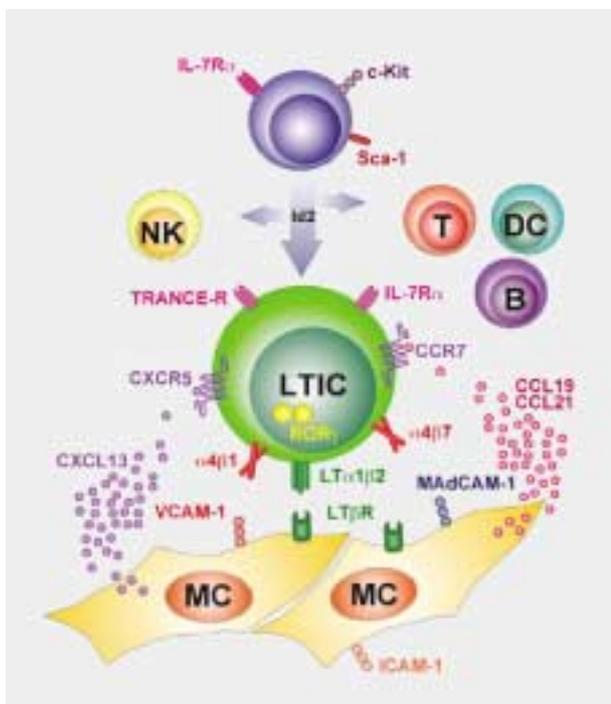
A murine model for Kaposi's sarcoma

(in cooperation with E. Kremmer, GSF; I. Anagnostopoulos, H. Stein, FU; K. Kölbl, Charité)

Infection with HHV-8 has been linked by epidemiological and molecular evidence to the pathogenesis of all forms of Kaposi's sarcoma (KS), a non-Hodgkin's B cell lymphoma, and multicentric Castleman's disease (MCD). The research project aims 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. Using a newly developed vGPCR-specific monoclonal antibody, significant expression of the viral chemokine receptor could be confirmed in all virus-associated human diseases.

The HHV-8-encoded vGPCR has been implicated in the pathogenesis of Kaposi's sarcoma particularly because of its high constitutive signaling activity. 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 immunocompetent BALB/c mice. Unexpectedly, vGPCR expressing cells established from grafted tumor fragments give rise to angioproliferative fibrosarcomas

Figure 1
Model for the role of CXCR5 and CCR7 during early steps of lymph node and Peyer's patch development. The accumulation of RORγt⁺ IL-7Rα⁺ lymphoid tissue inducer (LTI) cells, which derive from a population of fetal liver cells, and mesenchymal organizer (MC) cells is probably driven by a positive feedback loop. IL-7Rα⁺ LTI cells stimulated via IL-7Rα or TRANCE-R upregulate surface expression of LTα₁β₂. The stimulation of mesenchymal organizer cells in the developing lymph node or Peyer's patch anlage by IL-7Rα⁺ LTI cells via LTβR induces the expression of adhesion molecules, such as VCAM-1, ICAM-1, MadCAM-1 and chemokines, including CXCL13, CCL19, and most probably CCL21. In turn, CXCL13 and CCL21 leads to the activation of α₄β₁ or α₄β₇ integrin on LTI cells, which reinforces the interaction of inducer and organizer cells and facilitates continuous signaling through LTβR. The accumulation of LTI cells and their tight interaction with mesenchymal organizer cells eventually reaches a critical size or level of chemokine expression that allows lymph node and Peyer's patch development to proceed to the next stage which includes the immigration of other cell types such as B cells, T cells, and macrophages, as well as their organization into distinct microenvironments (Nat. Immunol., 5, 12-14, 2004).



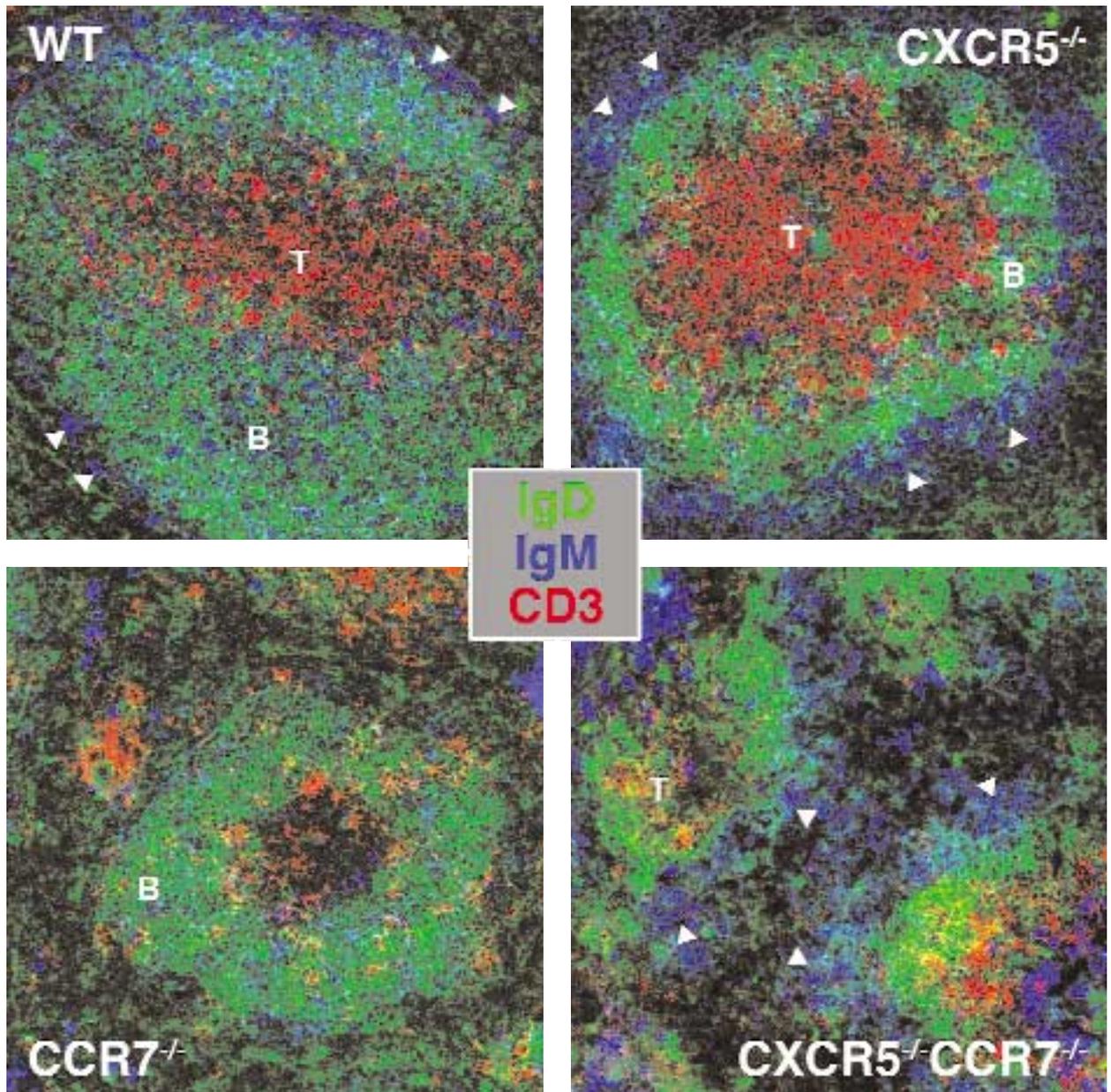


Figure 2

Altered primary B cell follicles in the spleen of CXCR5^{-/-}, CCR7^{-/-} and CXCR5^{-/-}CCR7^{-/-} mice. Cryostat sections were stained with PE-labeled anti-CD3 (red), FITC-labeled anti-IgD (green) and biotinylated anti-IgM/Streptavidin-Cy5 (blue) to visualize the architecture of primary B cell follicles. In wild-type mice, marginal zone B cells (IgM^{hi}IgD^{lo}) appear as a rim around the polarized clusters of follicular B cells, which are mainly IgM^{lo}IgD^{hi}. In CXCR5^{-/-} mice, B cells fail to organize in polarized clusters. The T cell zone is instead surrounded by a ring of IgM^{hi}IgD^{lo} B cells, which is in turn encompassed by a thickened ring of IgM^{hi}IgD^{lo} marginal zone B cells. CCR7^{-/-} mice generally lack prominent T cell zones in the white pulp. In contrast, T cell zones and B cell zones are largely disorganized in CXCR5^{-/-}CCR7^{-/-} mice. White arrows indicate the position of the IgM^{hi}IgD^{lo} marginal zone B cells; WT, wild type; T, T cell zone; B, B cell zone; Original magnification: x200 (Immunol. Rev. 195, 117-135, 2003)

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.

Function and signaling of Cytomegalovirus (CMV)-encoded chemokine receptor US28

(in cooperation with A. Rehm, J. Droese and B. Dörken)

The HCMV encoded receptor US28 may play an important role in the pathogenesis of herpesvirus infections through binding and sequestering of extracellular inflammatory β -chemokines. This project is aimed at elucidating the US28 signaling pathways underlying chemotaxis and chemokinesis in CMV infected cells. US28 displays constitutive activation of both phospholipase C and NF-kappaB signaling and exhibits a high basal level of phosphorylation independently from

ligand binding. We elucidated that such unique constitutive receptor phosphorylation is a prerequisite for the subcellular localization and for the rapid agonist-independent endocytosis of the receptor. In contrast to all other chemokine receptors, US28 was found to internalize in a clathrin-coated pit dependent, but arrestin-independent, manner. Currently, we are investigating the protective antiapoptotic pathways that may be employed by US28 expression to maintain survival of virus infected cells.

Role of sphingophospholipid receptors in the immune system

EDG receptors represent a novel family of G protein-coupled receptors, binding either lysophosphatidic acid (LPA) or sphingosine 1-phosphate (S1P). Five receptors, S1P₁ (EDG-1), S1P₂ (EDG-5), S1P₃ (EDG-3), S1P₄ (EDG-6), and S1P₅ (EDG-8), have been identified as high affinity receptors for S1P in mammals. S1P exerts a variety of responses, including proliferation, differentiation, and migration, by activating a distinct set of G proteins coupled to the S1P receptors in different types of cell. Our laboratory has identified and characterized S1P₄, which is expressed specifically in cells and tissues of the hematopoietic and lymphoid system. Recently, we have analyzed signaling pathways mediated via S1P₄ and have shown that S1P₄ couples directly to G α_i and very potently to G $\alpha_{12/13}$ -subunits of trimeric G-proteins. Consequently, S1P₄ induces pertussis toxin-sensitive PLC activation and Rho-GTPase-dependent cytoskeletal rearrangements such as peripheral stress fiber formation and cell rounding upon S1P stimulation. The capacity of S1P receptors to mediate fundamental responses such as cell motility and shape change through G α_i - and G $\alpha_{12/13}$ -coupled signaling pathways suggests an important *in vivo* role for the S1P system in the control of cell migration in the context of the tissue microenvironment in lymphocyte homeostasis and acute and chronic inflammatory immune responses. In order to explore the *in vivo* function of S1P receptors, S1P₄^{-/-} mice have been generated and are currently under investigation.

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Biology of Hodgkin's Lymphoma

Bernd Dörken

Scope

Hodgkin and Reed-Sternberg cells are tumor cells of classical Hodgkin's lymphoma. 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 try to identify molecular defects that might be responsible for tumor cell transformation.

Role of c-FLIP and AP-1 in the pathogenesis of Hodgkin's lymphoma

Stephan Mathas, Andreas Lietz, Franziska Jundt, Martin Janz in cooperation with Claus Scheidereit (MDC) and Harald Stein (Charité)

As a molecular defect responsible for proliferation and apoptosis resistance of Hodgkin-/Reed-Sternberg (HRS) cells, we identified a constitutive activity of transcription factor NF- κ B. In an attempt to establish inhibition of the NF- κ B activity as a treatment option for Hodgkin's lymphoma (HL), we were able to show a strong anti-tumor activity of arsenic containing compounds *in vitro* and *in vivo* in a mouse model. Furthermore, we could demonstrate that NF- κ B dependent up-regulation of c-FLIP proteins is the key mechanism for death receptor resistance of HRS cells, including inhibition of the CD95 and TRAIL receptor pathways. Ongoing work aims to identify the mechanisms leading to constitutive NF- κ B activity in HRS cells and to develop clinically applicable treatment options for HL based on NF- κ B inhibition. A further project of the group is the analysis of the recently described constitutive AP-1 activity in HRS cells. In this project, AP-1 activation mechanisms and target genes are investigated. This also includes the analysis of these transcription factors with respect to the dedifferentiation of HRS cells.

Characterization of disease-associated genes in Hodgkin's lymphoma

Martin Janz, Stephan Mathas in cooperation with Rudolf Manz (Deutsches Rheumaforschungszentrum), Christian Hagemeyer (Charité) and Harald Stein (Charité)

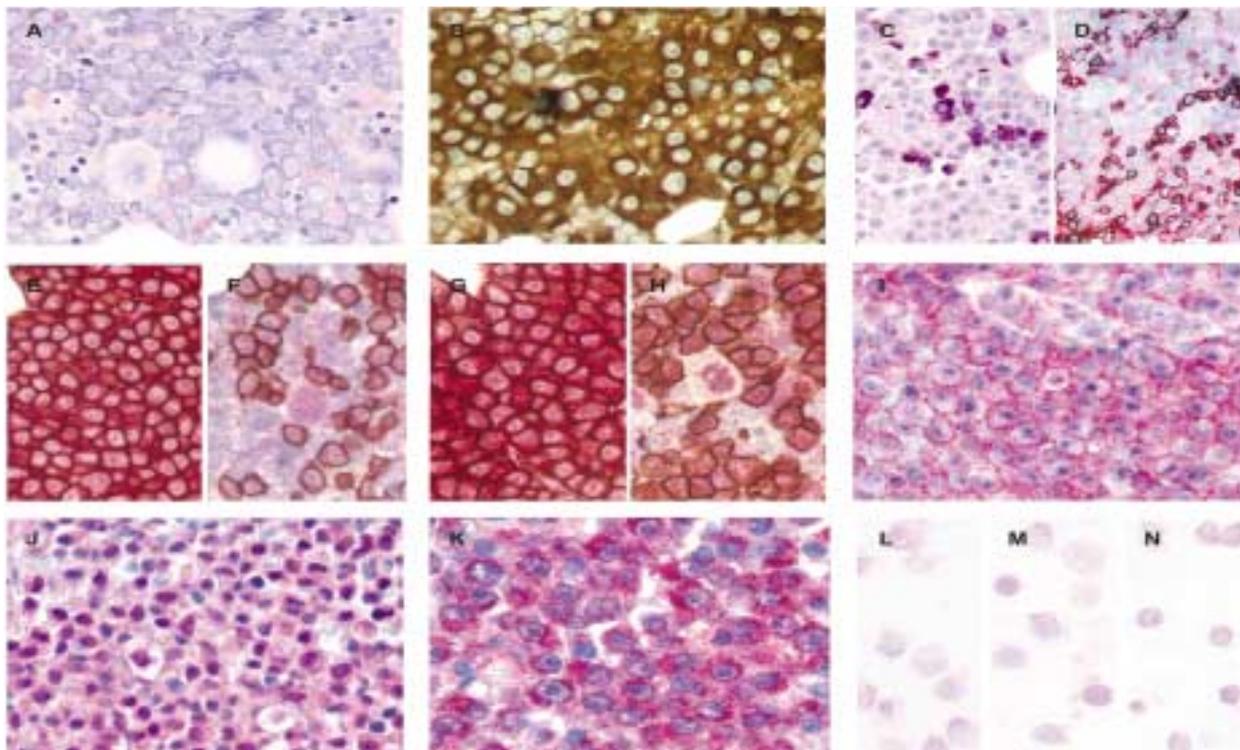
Malignant transformation of hematopoietic cells is associated with profound alterations in the transcriptional program resulting in deregulated proliferation, differentiation, and apoptosis. Using classical Hodgkin's lymphoma (cHL) as a model system, we are investigating the role of transcription factors in the oncogenic process. Using oligonucleotide microarrays, we have generated expression profiles for cHL-derived cell lines as well as Non-Hodgkin B-cell lines. In addition, we have analyzed the expression pattern of primary non-malignant human B- and T-cell populations. Our microarray data provide the basis for the identification of differentially expressed genes that determine the malignant phenotype of cHL. Candidate genes are further validated by *in situ* hybridization and immunohistochemistry on primary tumor tissue. This experimental strategy revealed that ATF3, a member of the CREB/ATF family of transcription factors that is involved in the cellular response to stress signals and has been shown to negatively regulate p53 function, is strongly overexpressed in primary Hodgkin cells. Using cell lines that constitutively overexpress ATF3, as well as vector-based siRNA constructs that downregulate ATF3 expression, we are investigating the significance of ATF3 in malignant growth and survival of lymphatic cells.

Biology of Hodgkin's lymphoma and multiple myeloma

Franziska Jundt, Kristina Schulze-Pröbsting, Katharina Kley in cooperation with Harald Stein (Charité)

Notch1 belongs to a family of transmembrane receptors that control cell proliferation and differentiation in response to extracellular ligands expressed on neighbouring cells. The Notch1 gene has been described as being involved in a translocation in a human acute T-cell lymphoblastic leukemia/lymphoma, and, its constitutively active form, produces T-cell neoplasms in mice. However, a pathogenetic role for Notch1 in B-cell neoplasms was so far unknown. We showed that Notch1 is strongly expressed in B-cell derived HRS cells and in tumor cells of T-cell derived anaplastic large cell lymphoma (ALCL). Our data indicate that activation of Notch1 signaling in tumor cells by its ligand Jagged1 regulates tumor cell growth and survival and suggest that pharmacological manipulation of the Notch1 system might have therapeutic potential in these lymphomas.

Furthermore, Notch 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 their bone marrow microenvironment, that are essential for tumor cell growth in multiple myeloma (MM). We demonstrated that Notch receptors and their ligand Jagged1 are highly expressed in cultured and primary MM cells, whereas non-neoplastic counterparts show low to undetectable levels of



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. (I, J) Some CD138 negative non-malignant bone marrow cells, mainly megacaryocytes, show weak immunoreactivity against Notch1 or Jagged1. (I-K) Extramedullary (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.

Notch. Functional data 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.

Identification of molecular regulators involved in B cell apoptosis

Barbara Tiedt, Ute Nitschke, Kurt Bommert in cooperation with Manfred Gossen (MDC)

Apoptotic downregulation of DNA replication is one of the possible mechanisms to ensure the fast and accurate execution of cell death. It might support the acceleration of DNA fragmentation and the saving of energy needed for the apoptotic processes. DNA replication itself is a highly organized and regulated process, which is initiated by binding of the pre-replicative complex (pre-RC) to the origins of DNA replication. Cdc6 and the MCM proteins (MCM2-7) are integral parts of the pre-RC. For two of the components, Mcm3 and Cdc6, we could show an apoptotic cleavage. The cleaving enzymes and the exact cleavage sites utilized in response to diverse apoptotic stimuli are analyzed for both proteins. The intracellular localization and chromatin association of wild-type Mcm3 and Cdc6 in comparison to caspase-resistant mutants and to the apoptotic protein fragments are analyzed. Furthermore, we could show a pro-apoptotic effect for one of

the apoptotic Mcm3 fragments, suggesting a self-enforcing mechanism by which proteolyzed caspase targets could contribute to the perpetuation of the apoptotic signaling cascade. We are now establishing the DT40 cell line model to test the function of the Mcm3 and Cdc6 fragments in a conditional knock out system.

Selected Publications

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Mechanisms of Immune Evasion in Tumor Biology and in Herpes Virus Infections

Armin Rehm (Helmholtz Fellow)

Scope

A major focus of our work is related to the role of immunosurveillance in tumor biology and Herpes virus infections. Viruses have evolved several mechanisms to employ their hosts' metabolic pathways to evade immune attack, also referred to as "stealth function". A second area of interest includes the mechanisms tumor cells employ to elude the immune system. We apply cell biological methods, as well as animal models, to study the pathophysiological role of a recently identified tumor-associated antigen shown to alter glycan structures at the cell surface of tumor cells.

Viral interference with the MHC class I antigen presentation pathway

Immunosurveillance is mediated by an innate immune response followed by the adaptive arm of the immune system for greater efficiency. Human cytomegalovirus encodes genes which inhibit MHC class I mediated antigen presentation. Among them, the US11 and US2 gene products dislocate efficiently MHC class I heavy chains out of the ER and into the cytosol where they are degraded by the proteasome. We have been able to show that the membrane glycoprotein US11 exhibits an unusual, delayed posttranslational signal peptide cleavage, which is presumably correlated with its immunoevasive function. US2 and US11 are not redundant but exhibit a significantly different efficiency in class I degradation when probed in human dendritic cells. We have generated transgenic mice that express their transferred genes in a tissue-specific, tetracycline inducible manner. This model will allow us to assess the proposed *in vivo* function of US11.

Role of the HCMV encoded chemokine receptor US28 in immune evasion

(in cooperation with U.E. Höpken, T. Mokros*, N. Lehmann, M. Lipp)

The HCMV encoded chemokine receptor, US28, serves as a decoy for inflammatory chemokines. We could show that rapid capture and endocytosis of chemokines relies on an agonist-independent phosphorylation at the intracellular C-terminus of the receptor. Receptor phosphorylation controls the rate of surface deposition, which is usually very low. In contrast to all other chemokine receptors, HCMV encoded US28 was found to internalize in a clathrin-coated pit dependent, but arrestin-independent manner. Furthermore, we could elucidate the structural motifs necessary for direct coupling of the receptor to the clathrin-coated pit associated adaptor molecules. Currently, we are exploring protective antiapoptotic pathways that may be employed in US28 expressing cells.

Physiological and pathophysiological role of the tumor-associated antigen RCAS1/ EBAG9

(in cooperation with U.E. Höpken and C. Birchmeier-Kohler)

Truncated O-linked glycan epitopes, among Tn and TF (Thomsen-Friedenreich), are a hallmark of essentially all types of experimental and human cancers. They are thought to be associated with cell adhesion, invasion, and metastasis of tumor cells.

We have identified a ubiquitously expressed Golgi-localized molecule, RCAS1/EBAG9, which modulates Tn and TF surface expression and was found to be expressed in high abundance in secretory active tumor tissues. The identification of an interaction partner points to a more physiological role in the secretory pathway, since the SNARE-associated molecule Snapin was identified. *In vivo*, we study the consequences of a genetic deletion of RCAS1/EBAG9 in a mouse knock out model where we focus on the generation of glycan epitopes, but also on the exocytosis function in endocrine and neuronal cells. Correspondingly, the modulatory role of RCAS1/EBAG9 *in vitro* in the exocytotic pathway is under scrutiny.

Association of lymphoma dissemination and chemokine receptor expression

(in cooperation with U.E. Höpken, M. Lipp, and I. Anagnostopoulos, UKBF)

The underlying mechanisms of migration and invasion of tumor cells often recapitulate those that are effective in non-neoplastic cells during physiological processes, among them immune-cell trafficking. It has been established that lymphocyte trafficking is largely orchestrated by the interaction of chemokine receptors and their cognate ligands. In this context, primary-mediastinal B-cell lymphoma are generally considered as a subgroup of diffuse large B cell lymphoma-however, they exhibit completely different patterns of dissemination and recurrence. Since they share a number of surface markers compared to thymic B cells, a close relationship was assumed. In this project, we take advantage of the chemokine/chemokine receptor system to assess functionally and

phenotypically the development of thymic B cells and we will correlate the profiles obtained from PMBL cell lines and tissues with their supposed ancestors. This approach will help to elucidate novel molecular markers for PMBL, but it might also help to define a pathogenetic trait of PMBL development.

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Identification of Target Structures for the Development of Molecular Therapies in Malignant B-cell Disorders

Ralf C. Bargou

Scope

The aim of our research is to identify target structures for the development of molecular therapies for malignant B-cell disorders. Therefore, we pursue two different strategies. The first strategy focuses on functional characterization of growth regulating signaling pathways in multiple myeloma in order to identify molecular targets for therapeutic intervention with small compound inhibitors. Another strategy focuses on the development of immunotherapies with recombinant antibody constructs for the treatment of B-cell Non-Hodgkin-lymphomas. Based on our preclinical research, we are conducting experimental phase I/II clinical trials.

Signaling and survival pathways in multiple myeloma

Dirk Hönemann, Manik Chatterjee, Suzanne Lentzsch, Ralf Bargou in cooperation with Reinhold Schäfer and Christine Sers (Institut of Pathology, Charité, Berlin)

The bone marrow microenvironment produces a number of different survival factors that are important for the malignant growth and drug resistance of multiple myeloma cells. One of the main factors reported to be important for survival and growth of myeloma cells is IL-6. However, very recently we could show that myeloma cells become independent of the IL-6-gp130-STAT-3 pathway if cells grow in the presence of their bone marrow microenvironment (BMM) suggesting that the BMM stimulates additional IL-6-independent survival pathways in multiple myeloma cells. Therefore, we are currently focusing on the identification and characterization of IL-6-independent pathways and IL-6-independent mechanisms of cell growth and survival. We have experimental evidence that Ras-triggered pathways, such as the MEK/MAPK and the PI3K/AKT pathway, are activated in myeloma cells by IL-6 independent mechanisms and contribute to the malignant phenotype of myeloma cells. Selective targeting of these pathways with either small compound inhibitors or siRNA constructs can induce cell death in myeloma cells.

Based on these studies, we plan to develop novel molecular therapeutic strategies.

Cytotoxic T-cell targeting by bispecific antibodies

Michael Grün, Anja Löffler, Ralf Bargou in cooperation with Gerd Riethmüller (München) and Patrick Baeuerle (Micromet GmbH, München)

We have shown that a novel recombinant bispecific single chain antibody, bscCD19xCD3, induces rapid and high lymphoma directed cytotoxicity mediated by unstimulated T-lymphocytes. By redirecting primary human T cells derived from the peripheral blood against CD19-positive lymphoma cell lines, the bscCD19xCD3 antibody showed significant cytotoxic activity at very low concentrations even in experiments without T cell prestimulation. In addition, the bscCD19xCD3 bispecific antibody is able to induce nearly complete depletion of primary lymphoma cells mediated by autologous T-cell of patients with chronic lymphatic leukemia in the majority of cases analyzed. Furthermore, we could demonstrate that this novel antibody construct is also effective in animal models. Based on these preclinical data, we have conducted a phase-I clinical trial for the treatment of patients with refractory B-cell lymphomas which was finished at the end of 2003. Another study (phase I/II) will start in 2004. Furthermore, we are currently establishing a similar strategy for the treatment of multiple myeloma patients using a novel plasmacell-specific surface antigen as a target structure.

Selected Publications

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

Wolf-Dieter Ludwig

Scope

Recent and ongoing research projects of the Acute Leukemia Research Group have investigated cell-biological determinants and mechanisms mediating drug resistance in leukemic blasts as well as their prognostic impact in acute leukemias. These research projects have been embedded in the framework of internationally renowned multicenter trials in childhood (ALL-BFM) and adult (AML CG) acute leukemias, thereby providing the opportunity to investigate biologically and clinically well-characterized leukemic specimens, and to correlate biologic findings with treatment response and prognosis. We have especially focused on mechanisms of regulation/deregulation of apoptosis, on detection of minimal residual disease (MRD), and on characterization of MRD cells using global gene expression profiling in acute leukemias.

Identification of apoptosis-related cell-biological determinants associated with therapy response

Deregulated or deficient activation of apoptosis signaling pathways may account for treatment failure in acute leukemias. Given the dramatically different clinical courses of patients with precursor T-cell acute lymphoblastic leukemia (ALL), we have been interested in apoptosis regulation in a large series of children with chemoresistant or chemosensitive disease and analyzed their susceptibility to cytotoxic drug-induced apoptosis, cytokine responsiveness, and cytokine modulation of apoptotic pathways induced by different drugs. Evaluation of these data in the context of immunophenotype and clinical data revealed that precursor T-cell ALL comprises several biologically distinct diseases and pointed to a predictive value of *in vitro* apoptosis-related functional parameters for treatment response *in vivo*. In particular, our studies revealed maturation-dependent differences in the regulatory pathways of apoptosis, suggesting that patients with a cortical thymocyte immunophenotype are much more responsive to drug-induced apoptosis as compared with patients exhibiting an immature or mature immunophenotype.

To investigate the key events of apoptosis at the mitochondrial level in primary leukemia cells, we developed a flow-cytometric method for simultaneous detection of mitochondrial cytochrome *c* release and caspase-3 activation, using a conformation-sensitive cytochrome *c* antibody and antibodies specifically detecting the cleaved fragment of caspase-3. The method proved to detect deficient mitochondrial apoptosis signaling in leukemic blasts from pediatric ALL patients, and identified the cytochrome *c* mediated caspase activation as the most predictive parameter for blast cell persistence on day 15 of induction therapy. These data indicate that constitutive differences in the activation of post-mitochondrial apoptosis signaling pathways significantly determine the initial response to treatment in childhood ALL (in cooperation with K.-M. Debatin, University of Ulm).

Identification of leukemia-associated immunophenotype (LAIP) of MRD cells and treatment response assessment in acute leukemia

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 LAIP of MRD cells. We are now focusing on validation of this panel within the framework of ongoing European multicenter clinical trials in childhood ALL and on its application in the molecular characterization of MRD cells by global gene expression analysis (see below). (in cooperation with: M. Dworzak, St. Anna Children's Hospital Vienna, A. Biondi and G. Gaipa, University of Milano, G. Basso, University of Padua)

Gene expression analysis of leukemic blasts persisting during induction therapy

Current studies on global gene expression in childhood ALL have analyzed a rather heterogeneous bulk of ALL blasts and do not enable the retrieval of specific information on treatment-induced gene expression changes in resistant or relapsed leukemic subclones, which may be "hidden" behind the bulk of chemotherapy-sensitive leukemic blasts. Therefore, we have chosen an alternative strategy aimed at direct investigation of gene expression in leukemic blasts persisting under prednisone on day 8 (i.e., prednisone poor response), a parameter with crucial prognostic significance in childhood ALL. To this end, an experimental approach has been established with sample acquisition and identification of MRD blasts based on their leukemia-associated immunophenotype as defined by multi-parametric flow cytometry. The approach has further included isolation of leukemic blasts by flow sorting, providing high-quality mRNAs. Most importantly, we have demonstrated that this experimental approach facilitates gene expression profiling of patient samples with blast cell counts as low as 50-100 cells/ μ l, thus even enabling investigation of patients with good response to prednisone. Intraindividual comparative analyses of matched "day 8" and "day 0" ALL samples revealed a set of

genes whose expression has been commonly changed in the “day 8” leukemic blasts. The observed changes have indicated a deregulated expression of certain signaling (e.g. STAT1, MAP3K8, CD13R)- and drug sensitivity (e.g. ABCA7)-related genes and suggested a preferential positioning of these cells in the early G1 cell cycle phase. These data illuminate the potential of this strategy to investigate treatment-specific gene expression changes and to identify molecular targets in treatment-resistant ALL blasts (in cooperation with C. Hagemeier, K. Seeger, Charité, Berlin CancerNet)

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Role of Apoptosis and Senescence in Tumor Development and Treatment Responses

Clemens A. Schmitt

Scope

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, for example, may not only facilitate tumor development, but might simultaneously co-select for resistance to anticancer therapy as well. Dissecting the pathways that control and execute apoptosis and senescence is expected to identify critical regulators that may be targeted by novel therapies.

Dissecting p53 tumor suppressor functions in vivo

We previously demonstrated that loss of p53 function accelerates tumor development and impairs sensitivity to anticancer therapy in vitro and in vivo. Although the p53 tumor suppressor acts in a plethora of processes that influence cellular proliferation and survival, it remains unclear which p53 functions are essential for tumor suppression and, as a consequence, are selected against during tumor development. Using a mouse model harboring primary, genetically-modified myc-driven B cell lymphomas, we show that disruption of apoptosis downstream of p53 by Bcl2 or a dominant-negative caspase 9 by retroviral modification of hematopoietic stem cells confers – like p53 loss – a selective advantage, and completely alleviates pressure to inactivate p53 during lymphomagenesis. Despite their p53-null-like aggressive phenotype, apoptosis-defective lymphomas that retain intact p53 genes do not display the checkpoint defects and gross aneuploidy that are characteristic of p53 mutant tumors. Therefore, apoptosis could be identified as the only p53 function selected against during lymphoma development, whereas defective cell-cycle checkpoints and aneuploidy are mere byproducts of p53 loss.

Consequently, we are currently testing to what extent individual cancer-derived p53-missense mutants and defects in the p53 activating DNA damage signaling cascade may produce biased decisions towards distinct p53 controlled effector functions such as apoptosis or cellular senescence prior to and after DNA damaging therapies.

p53 and p16^{INK4a} control cellular senescence as a drug-responsive program that impacts on the outcome of cancer therapy

As previously reported, p53 and INK4a/ARF mutations promote tumorigenesis and drug resistance, in part, by disabling apoptosis. We now show that primary murine lymphomas also respond to chemotherapy by engaging a senescence program controlled by p53 and p16^{INK4a}. Hence, tumors with p53 or INK4a/ARF mutations – but not those lacking ARF alone – respond poorly to cyclophosphamide therapy in vivo. Moreover, tumors harboring a Bcl2 mediated apoptotic block undergo drug-induced cytostasis involving the accumulation of p53, p16^{INK4a}, and senescence markers, and typically acquire p53 or INK4a mutations upon progression to a terminal stage. Finally, mice bearing tumors capable of drug-induced senescence have a much better prognosis following chemotherapy than those harboring tumors with senescence defects. Therefore, cellular senescence contributes to treatment outcome in vivo.

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.

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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. The molecular basis of these events has been studied extensively during recent years and comprehensive models are now established for large parts of these signaling events. Studies utilizing both *in vitro* and *in vivo* models in genetically defined cell line models or knock-out mice clearly demonstrated that inactivation of p53 (or of genes acting in the same signaling cascade, i.e. the p53 pathway) may result

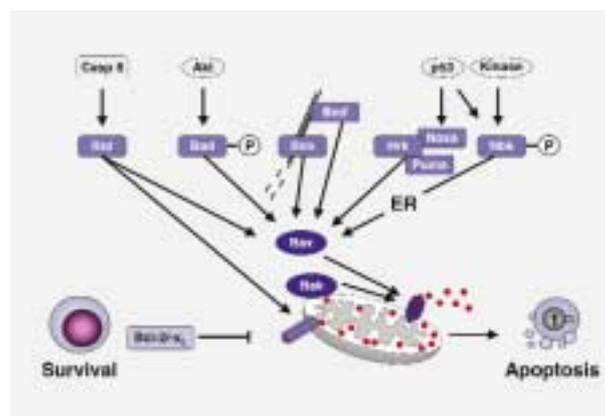
in resistance to anticancer therapy. In theory, the inactivation of p53 should also result in clinical resistance. Nevertheless, the clinical consequences of p53 disruption in human cancer have been discussed controversially. While there is few doubt that p53 disruption can serve as a key step during tumorigenesis, 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.

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 (Figure 1). Cleavage of Bid by caspases-8 or -3, e.g. during death-receptor-induced apoptosis generates tBid that

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



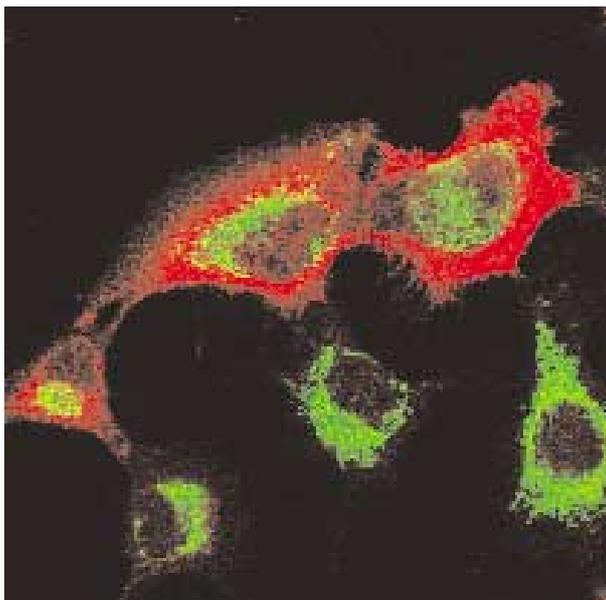


Figure 2 Immunocytochemistry for NbK localisation in DU145 prostate carcinoma. Red: NbK (localizes to the ER), green: mitochondria.

binds to Bax. This triggers a conformational switch in Bax that exposes the Bax C-terminus, induces redistribution of Bax to the mitochondria, and insertion into the outer mitochondrial membrane. In contrast, Bak is localized constitutively in the outer mitochondrial membrane. Upon activation, both Bax and Bak oligomerize and form channels that release cytochrome c. Bim is released from the motor-dynein complex whereas Bmf is released from actin-myosin filaments during apoptosis. Puma, Noxa, Hrk, and NbK (Bik) are induced by p53 and mediate cell death originating from the nucleus, e.g. upon DNA damage. Phosphorylation of NbK by a so far undefined kinase enhances NbK function. NbK localizes to the ER (Figure 2) and activates Bax indirectly, through a so far undefined ER-initiated death pathway (Gillissen et al., EMBO J 2003; 22:3580-90). In contrast, phosphorylation of Bad through the Akt/PKB kinase inactivates Bad. The latter disrupts the binding of Bad to Bcl-xL and results in Bax and Bak-dependent apoptosis.

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

Selected Publications

Gillissen B, Essmann F, Graupner V, Stärck L, Radetzki S, Dörken B, Schulze-Osthoff K, Daniel PT (2003) Induction of cell death by the BH3-only Bcl-2 homolog NbK/Bik is mediated by an entirely Bax-dependent mitochondrial pathway. *EMBO J*; 22:3580-90.

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Molecular Immunotherapy

Antonio Pezzutto

Our group focuses on four topics: 1) Use of dendritic cells to induce tumor-specific immune response and identification of target antigens for immunotherapy by mass spectrometry analysis of MHC-bound peptides isolated from tumor cells; 2) Analysis of the natural immunity against the adenocarcinoma-associated EpCAM antigen in cancer patients and vaccination in EpCAM transgenic mice; 3) Evaluation of gene transfer and DNA vaccination for induction of tumor specific immunity; and 4) Evaluation of graft versus leukemia effects in murine models of bone marrow transplantation.

Use of dendritic cells for the induction of anti-leukemic immune response

Jörg Westermann, Monika Schwarz, and Tuan Duc Nguyen in cooperation with E-M. Müller and Albrecht Otto (MDC group of Proteomics).

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. To boost the leukemia specific immune response in patients, we perform clinical vaccination studies using in vitro-generated, bcr-abl positive dendritic cells (DC). Using tandem mass spectrometry (MS/MS) on peptides that are naturally presented and eluted from MHC-I molecules of leukemic cells, we have identified peptides that appear to identify tumor associated antigens and are potential candidates for the development of vaccination strategies (cooperation project with E-M. Müller and A. Otto, (MDC Group of Proteomics). Two HLA-A3 binding peptides from the LMO-4 and the LYL-1 proteins appear to be particularly interesting. LMO-4 is the most recently identified member of a gene family of "Lim-only" transcription factors that has been reported to be increased in breast cancer, prostate cancer, and some acute T-cell leukemia cells. LYL-1 belongs to a subgroup of Helix-Loop-Helix proteins that have been claimed to play a role in the development of acute T-cell leukemia. The technique for analysis of MHC-bound peptides by mass spectrometry is being extended to other cancer types.

Induction of T-cell immunity against EpCam (Epithelial Cell adhesion molecule)

Oliver Schmetzer in cooperation with T. Kammertöns (MDC group of Molecular Immunology and Gene Therapy), P. Schlag (MDC group of surgical oncology) and K. Falk and O. Röttschke (MDC group of cellular immunology of autoimmune reactions).

Some patients with colorectal cancer develop an immune response against peptides of the epithelial adhesion molecule EpCam, which is overexpressed in human adenocarcinomas. A sensitive ELISA assay for the screening of naturally occurring autoantibodies against EpCam, as well as circulating EpCam protein, has been established. Induction of autoantibodies against EpCam is an infrequent event occurring only in a minority of patients with adenocarcinomas (cooperation with P. Schlag). Some patients have circulating CD4 positive T cells that recognize MHC-II binding EpCam peptides. Since multimeric 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. Röttschke. Transgenic mice expressing human EpCam have been generated for use in preclinical vaccination experiments (cooperation with T. Kammertöns, and Th. Blankenstein.) A clinical vaccination study in patients with EpCam-positive adenocarcinomas is in preparation.

DNA Vaccination

Jörg Westermann, Tam Nguyen Hoai in cooperation with U. Höpken and M. Lipp (MDC group of Differentiation and growth control in lymphocyte development).

In order to increase the delivery of genes to antigen presenting cells, we have evaluated targeting of DNA-Polyethylenimine-Mannose complexes to dendritic cells via the mannose receptor. We have found that the cytokine Flt-3 Ligand (which can induce in vivo expansion and recruitment of dendritic cells) used together with DNA vaccination is not able to induce a TH1 polarized immune response. In cooperation with the MDC group of M. Lipp (Molecular Tumor Genetics), we are exploring the possibility of recruiting immune cells at the vaccine site by inserting DNA sequences coding for specific chemokine/chemokine receptors during DNA vaccination.

Exploitation of the Graft versus Leukemia effect in the context of allogeneic stem cell transplantation.

Corinna Leng and Markus Y. Mapara

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 in which immune effector cells of the donor (primarily T lymphocytes and to a lesser extent Natural Killer (NK) cells) recognize the recipient as "foreign" and attack the recipient's tumor cells (e.g. leukemia or lymphoma cells). Its widespread application, however, has been precluded by the development of graft-versus-host-disease (GVHD), which can lead to

Molecular Immunology and Gene Therapy

Thomas Blankenstein

In the last two years, the group made several discoveries related to the question of how the immune system rejects tumors. Importantly, the role of the tumor stroma as a target for immune mediated tumor rejection was investigated. Additionally, a new mechanism was discovered showing that chemical carcinogenesis can be spontaneously controlled in an immunological manner. Examples of the group's activities during the report period are given below.

Tumor Rejection by Disturbing Tumor Stroma Cell Interactions

The stroma of solid tumors is a complex network of different cell types. Little is known about the stroma cell interactions in a permissive or hostile tumor environment. We analyzed such interactions in growing tumors and during their rejection. Tumor infiltrating macrophages (TIM) require T cells to become effector cells but, in growing tumors, are committed to produce IL-10. Following T cell inactivation, TIM immediately switch to Interferon- γ (IFN γ) production and the tumor vasculature is destroyed in an IFN γ -receptor-dependent fashion. These events precede hemorrhagic necrosis and residual tumor cell elimination by T cells. Together, T cells suppress their environment through regulation of macrophage function and tumor rejection can be induced by disturbing the tumor stroma network.

Generation of tumor-associated cytotoxic T lymphocytes requires IL-4 from CD8⁺ T cells

Activation of tumor-associated CD8⁺ cytotoxic T lymphocytes (CTLs) often requires antigen re-presentation, e.g. by dendritic cells (DCs), and CD4⁺ T cell help. Previously, we showed that CTL-mediated tumor immunity required interleukin-4 (IL-4) during the immunization but not effector phase. In order to determine the source and target cells of IL-4, we performed adoptive T cell transfers using CD4⁺ and CD8⁺ T cells from IL-4^{-/-} and IL-4R^{-/-} mice and analyzed

CTL generation. Even though necessary for CTL generation, CD4⁺ T cells did not need to express IL-4 or IL-4R. Surprisingly, CTL generation required IL-4 but not IL-4R expression by CD8⁺ T cells. Since IL-4 (i) was expressed by naive CD8⁺ T cells within 24 h after antigen-encounter, (ii) IL-4 induced DC maturation and (iii) CTL development was impaired in T cell-reconstituted IL-4R^{-/-} mice, CD8⁺ T cell-derived IL-4 appears to act on DCs. We conclude that CD4⁺ and CD8⁺ T cells provide different signals for DC activation during CTL generation.

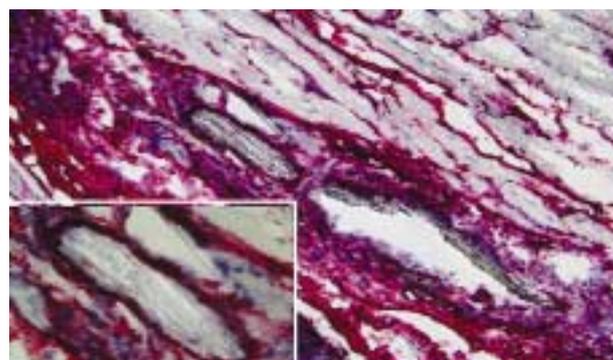
Chemical carcinogenesis is inhibited by an IFN γ -receptor-dependent foreign body reaction

The foreign body reaction is one of the oldest host defense mechanisms against tissue damage which involves inflammation, scarring, and encapsulation. The chemical carcinogen methylcholanthrene (MCA) induces fibrosarcoma and tissue damage in parallel at the injection site. Tumor development by MCA, but not p53-deficiency, is increased in interferon- γ receptor (IFN γ R) deficient mice. In the absence of IFN γ R, MCA diffusion and DNA damage of surrounding cells are increased. Locally produced IFN γ induces the formation of a fibrotic capsule. Encapsulated MCA can persist virtually life-long in mice without inducing tumors. Together, the foreign body reaction against MCA prevents malignant transformation, probably by reducing DNA damage, and it is more efficient in the presence of IFN γ R. This indicates that inflammation and scarring, both suspected to contribute to malignancy, are protective during chemical carcinogenesis.

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 major histocompatibility class (MHC) I molecules. In addition, tumor-derived antigens can be cross-presented to CD8⁺ effector T cells by antigen-presenting cells (APCs). Interferon- γ (IFN γ) production by CD8⁺ T cells is often critical for tumor rejection. However, it remained unclear as to

An IFN γ -supported foreign body reaction protects from chemical carcinogenesis (methylcholanthrene). Encapsulated MCA persists within fibrotic area in long-term tumor-free mice. Shown is the staining with mAb ER-TR7 of a muscle section of a tumor-free IFN γ -R-WT mouse injected more than a year ago with MCA. The inset figure shows a piece of encapsulated MCA at higher magnification. Data are from Qin et al. (2002).



whether (i) CD8⁺ T cells secrete IFN γ in response to antigen recognition on tumor cells or APCs and (ii) whether IFN γ mediates its anti-tumor effect by acting on host or tumor cells. We showed that CD8⁺ effector T cells can reject tumors in bone marrow (BM) chimeric mice incapable of cross-presenting antigen by BM-derived APCs and that tumor rejection required host cells to express IFN γ receptor. Together, CD8⁺ effector T cells recognize antigen directly on tumor cells which is sufficient to reject tumors by IFN γ acting on host cells.

A Critical Requirement of Interferon Gamma-Mediated Angiostasis for Tumor Rejection by CD8⁺ T Cells

It is thought that tumor rejection by CD8⁺ T cell effectors is primarily mediated by direct killing. We show that rejection of different tumors (fibrosarcoma, ras-transformed fibroblasts, colon carcinoma, plasmacytoma) by CD8⁺ T cells is always preceded by inhibition of tumor-induced angiogenesis. Angiostasis and tumor rejection were observed in perforin, but not in IFN γ -deficient mice. Furthermore, adoptive transfer of tumor-specific CD8⁺ T cells from IFN γ -competent mice inhibited angiogenesis of lung metastases in comparison to those from IFN γ -gene deficient mice. Together with our previous findings, we conclude that IFN γ dependent anti-angiogenesis is a general mechanism involved in tumor rejection by CD4⁺ and CD8⁺ T cell effectors.

Tumor rejection by modulation of tumor stromal fibroblasts

IL-4-secreting tumors are rejected in mice, an effect that is thought to be immune-mediated. However, solid tumors are embedded in a stroma that often contains tumor-promoting fibroblasts, a cell population whose function is also affected by IL-4. We showed that IL-4-secreting tumors grew undiminished in IL-4 receptor-deficient (IL-4R^{-/-}) mice. In IL-4R^{+/+} mice, they were long-term suppressed in the absence of T cells but complete rejection required T cells, compatible with the assumption that hematopoietic cells needed to respond to IL-4. Surprisingly, bone marrow (BM) chimeric mice revealed that IL-4R expression exclusively on non-BM-derived cells was sufficient for tumor rejection. Fibroblasts in the tumor stroma were identified as a target cell type for IL-4, since they accumulated in IL-4-secreting tumors and displayed an activated phenotype. Additionally, co-injection of IL-4R^{+/+}, but not of IL-4R^{-/-}, fibroblasts was sufficient for the rejection of IL-4-secreting tumors in IL-4R^{-/-} mice. Our data demonstrate a novel mechanism by which IL-4 contributes to tumor rejection and show that the targeted modulation of tumor-associated fibroblasts can be sufficient for tumor rejection.

Selected Publications

Qin, Z., Schwartzkopff, J., Pradera, F., Kammertöns, T., Seliger, B., Pircher, H. and Blankenstein, Th. (2003). A criti-

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Molecular Cell Biology and Gene Therapy

Wolfgang Uckert

The group addresses questions related to the optimization of viral vectors for gene transfer and the generation and transfer of genetically modified T cells in tumor therapy.

Retroviral vectors derived from Moloney murine leukemia virus are commonly used to transfer foreign genetic information into T cells. We showed that retroviral vectors carrying the envelope of murine leukemia virus 10A1 more efficiently transduce human primary T cells in comparison to vectors pseudotyped with the envelope of other retroviruses. However, when using these vectors, the expression level of transferred genes is often unsatisfactory. We analyzed different *cis*-regulatory elements and constructed retroviral vectors (MP71) that yielded an up to 75-fold increase of transgene expression in T cells in comparison with those elements used in murine leukemia virus based vectors.

Using an MP71 vector, the α and β chains of a T cell receptor (TCR), that recognizes a tumor-associated antigen on renal cell carcinoma cells, was transferred into T cells. The TCR molecule was expressed on the cell surface of human primary T cells of different donors. TCR gene-modified T cells could be stimulated in an antigen-specific, HLA-restricted fashion and led to the recognition and lysis of renal cell carcinoma cells, comparable to T cell clones originally isolated from the tumor. Thus, the redirection of T cells by TCR gene transfer could greatly facilitate adoptive therapy.

However, little is known about how two TCRs on one T cell influence antigen recognition, specificity, and tumor cell lysis. Therefore, we have generated a mouse model of double TCR T cells (OT-I x P14), specific for ovalbumin (ova) and LCMV glycoprotein 33 (gp33). These cells retain both specificities and can be activated by their respective 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 *in vitro* T cell activation.

In a further project, we developed and analyzed recombinant adenoviruses as gene transfer vectors. We evaluated the therapeutic potential of these vectors in animal experiments and analyzed possible reasons for side effects in clinical studies. We showed complement activation in isolated plasma of patients that harbored antibodies directed against adenoviral antigens after challenge with recombinant adenoviruses. Furthermore, we found an increased specific affinity of the commonly used adenovirus serotype 5 (Ad5) and human erythrocytes leading to hemagglutination. This phenomenon is of importance with regard to the calculation of organ-specific gene transfer efficacies and may be linked to other pathophysiological reactions. Moreover, we showed that the use of constitutive nonregulated promoters, that are commonly used in adenoviral vectors, lead to a metabolic overload of transgenic cells.

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M. Mühlebach, I. Schmitt, S. Steidl, J. Stitz, M. Schweizer, T. Blankenstein, C. Cichutek, W. Uckert. Transduction efficiencies of MLV-, but not of HIV-1-vectors are pseudotype-dependent on human primary T lymphocytes. *J. Mol. Med.* 81: 801-810 (2003).

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Molecular and Cell Biology of Hematopoietic Cells

Martin Zenke

Hematopoietic cells develop from a population of hematopoietic stem cells in bone marrow through multiple steps of lineage commitment and differentiation. The focus of research of this group is the molecular and cell biology of two hematopoietic cell types: red blood cells and antigen presenting dendritic cells. Their development from stem/progenitor cells is studied in human cells and, in experimental model systems, in hematopoietic cells of mouse and chicken. Emphasis is put on the identification of genes that determine lineage fate and that will eventually allow to directly influence cell fate and to generate cells with wanted properties.

bFGF signaling, Myb, and GATA-1 transcription factors in red cell development

Bartunek, P. and Blendinger, G.

Red blood cells represent one of the most abundant specialized cell types in vertebrate organisms. They develop from hematopoietic stem cells through successive steps of differentiation, including progenitors that are already committed to the red cell lineage. We have now identified an erythroid progenitor that is critically dependent on bFGF and requires expression of ν -Myb for sustained proliferation in vitro (Bartunek et al., 2002; in collaboration with M. Dvorak, IMG, Prague). In the presence of bFGF such ν -Myb cells are completely blocked in their ability to differentiate and exhibit an exceptionally high proliferative potential and long lifespan in vitro. In the absence of bFGF cells effectively differentiate into mature erythrocytes, irrespective of constitutive and elevated levels of ν -Myb. Our studies suggest that bFGF, in cooperation with Myb protein, represents an important factor for determining erythroid lineage choice.

Our previous work demonstrated that GATA-1 and GATA-2 transcription factors are important for red cell development (Briegel et al., *Genes & Dev.* 7, 1097-1109, 1993; Briegel et al., *Development* 122, 3839-3850, 1996; and references therein). We have employed a culture system that faithfully recapitulates red blood cell differentiation in vitro, to follow

the kinetics of GATA-1 and c-Myb expression. We show that c-Myb expression is high in progenitors and effectively downregulated when GATA-1 is induced and cells differentiate into erythrocytes (Bartunek et al., 2003; in collaboration with M. Dvorak, IMG, Prague). We also found that GATA-1 regulates c-Myb through binding to two GATA-1 sites in the c-myb promoter and this requires FOG-1. Thus, our study provides a direct molecular link between GATA-1 activity and c-myb expression during terminal red cell differentiation.

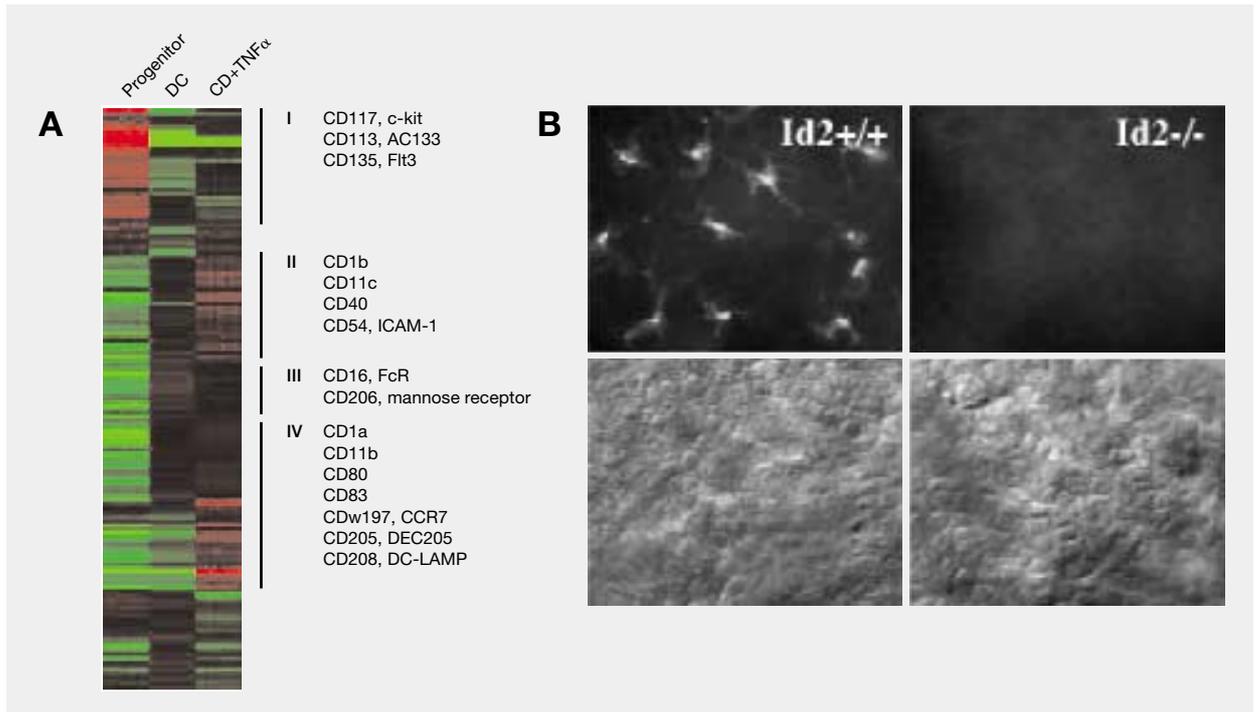
Transcriptional profiling by DNA microarrays identifies Id2 function in dendritic cell development

Hacker, C., Ju, X.-S., Hieronymus, T., Kirsch, R. D., Kurz, S. M., Jorgas, T., Knespel, S., Euler, U.

Dendritic cells (DC) are professional antigen presenting cells that play key roles in antigen specific T cell responses and have been implicated in determining the balance between immunity and tolerance. DC originate from hematopoietic progenitor cells and occur throughout the organism, both in lymphoid and nonlymphoid tissues, and are specialized for uptake, transport, processing, and presentation of antigens. Unique DC subclasses have been identified that differ in phenotype, function, activation state, and location but their relationship and developmental origins have remained unclear or controversial.

To study DC development, in vitro systems for differentiation of human and mouse DC from hematopoietic stem/progenitor cells were developed (Hacker et al., 2003; Ju et al., 2003; Hieronymus et al., submitted). In these culture systems, cells are grown with a stem cell factor/cytokine cocktail that maintains the progenitor phenotype and cells are induced to undergo synchronous differentiation into DC by administration of GM-CSF and IL-4. Transcriptional profiling with microarrays was used to determine the transcription factor repertoire of DC. This study identified the inhibitory helix-loop-helix transcription factor Id2 as one of the most prominently increased factors during DC development in vitro (Hacker et al., 2003). By studying Id2^{-/-} mice we demonstrate that Id2 is crucial for development of distinct DC subsets in vivo. Id2^{-/-} mice lack Langerhans cells (LC), the cutaneous contingent of DC, and a specific splenic DC subset. TGF β ^{-/-} mice also lack LC and we show that TGF β acts upstream of Id2 and induces Id2 expression.

Id2^{-/-} mice have higher B cell numbers than Id2^{+/+} mice and we found that Id2 represses B cell genes in DC (Hacker et al., 2003). Thus, a model presents itself where the relative expression levels of Id2 and activating HLH factor, such as E2A, determine lineage choice by affecting the propensity of such a common progenitor to develop into B cells or DC: low or no expression of Id2 and high expression of E2A supports B cell development while high expression of Id2 blunts E2A activity and B cell development, and thus allows differentiation into DC.



(A) Hierarchical cluster analysis of DNA microarray data of DC progenitor cells (progenitor) and differentiated DC (DC); DC+TNF α , differentiated DC treated with TNF α for an additional 16 hours. Green, low expression; black, intermediate expression; red, high expression (for details see Hacker et al., 2003).

(B) *Id2*^{-/-} mice lack Langerhans cells. Immunofluorescence analysis of epidermis of *Id2*^{-/-} and *Id2*^{+/+} mice for MHC class II expression (top panel). Lower panel, phase contrast images of the respective fields in top panel (Hacker et al., 2003).

Gene transfer into antigen presenting dendritic cells (DC)

Gust, T. C. and Schröder, D.

Given their unique properties in antigen specific T cell activation, DC represent a particularly attractive cell type for use in immunotherapy of diseases. Following our interest in generating gene modified DC by receptor mediated endocytosis (Diebold et al., Hum. Gene Ther. 10,775-786, 1999; Diebold et al., Gene Ther. 8, 487-493, 2001), we have now further developed our gene delivery systems for DC. DC are post-mitotic and notoriously difficult to transfect with DNA. Accordingly, adenovirus polyethylenimine (Ad/PEI) RNA transfer complexes were generated that contain adenovirus particles as ligand and in vitro transcribed RNA condensed by PEI (Gust et al., 2004). These Ad/PEI/RNA complexes were found to be more effective in transducing DC and in inducing antigen specific T cell responses than DNA containing complexes. This is because transfected RNA readily reaches the cytosol and is translated, thus obviating the need for nuclear translocation.

Selected Publications

Bartunek, P., Kralova, J., Blendinger, G., Dvorak, M. and Zenke, M. (2003). GATA-1 and c-myb crosstalk during red blood cell differentiation through GATA-1 binding sites in the c-myb promoter. *Oncogene* 22, 1927-1935.

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Cellular Immunology of Autoimmune Reactions

Kirsten Falk
Olaf Röttschke

The driving force in the progression of autoimmune diseases are the 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 as 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) Environmental factors for the induction of autoimmune reactions

It is already well established that genetic factors, such as expression of certain allelic forms of MHC class II 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 demonstrated that certain small molecular compounds are able to trigger the exchange of peptide antigens on the surface of activated antigen presenting cells. These antigens include autoantigens (peptides and proteins), the target structure of autoreactive T cells. Studies by other groups have shown that peptides derived from these autoantigens can induce fatal autoimmune reactions when loaded onto activated dendritic cells. By catalysing this process, these small molecular compounds might, therefore, represent environmental risk factors which have not been considered previously. On the other hand, these compounds could also be applied in therapeutic settings. For instance, by mediating the transfer of tumor-specific antigens, they can enhance immune responses directed against the transformed tissue. The group is currently defining the structural requirements of the compounds and investigates their impact on several experimental autoimmune and tumor model systems.

2) Control of autoimmune reactions

Several mechanisms have been described which potentially allow the control of 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) and the heat shock protein gp96, which had been previously found to be effective tolerance inducers *in vivo*. Primary goals of these studies are the exploration of ways allowing for 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 can be fatal, it can be beneficial in the context of tumour-immunotherapies. In contrast to other 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 CD8⁺ CTL or B cells, which support or continue the tissue-specific removal of cells. In our group, initial tests are being carried out in which the capacity of autoreactive CD4⁺ T cells is tested with samples from tumour patients and with experimental mouse model systems. The trials employ antigens with enhanced immunogenicity, such as epitope oligomers or lipopeptides, 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 immunoregulation) are implemented in these trials to achieve additional leverage by generating proinflammatory environments promoting tumor-specific immune responses.

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Translational research, bridging the gap between fundamental and clinical research, is the main research focus of our group. Using preferably in vivo mouse models, we are interested in investigations concerning novel therapeutic approaches for the treatment of cancer. Recent research dealt with three main topics:

- Characterization of patient-derived leukemias and colon carcinomas for clinical relevance
- Testing of in vivo procedures for gene therapy
- Stem cell engraftment and proliferation

Clinical relevance of xenotransplanted malignancies

It is essential that pre-clinical models used to predict the activity of novel therapeutic strategies for the treatment of cancer coincide in decisive parameters with the clinical phenotype. Therefore, we were interested in the establishment of xenografts directly from patient material into immunodeficient mice. In vivo transplantable lines were maintained in early passages and characterized in terms of their relation to the original samples.

We could show that, after several murine passages, acute lymphoblastic leukemias (ALL) still resembled the clinical disease in terms of both immuno- and genotype but also in relation to chemoresponsiveness. ALL established from primarily diagnosed patients revealed a higher response to anti-leukemic drugs than leukemias from relapsed patients. The treatment success was conversely related to the expression of resistance markers (P-gp, LRP, MRP1) on the leukemic cells.

Studies performed in close cooperation with the Robert-Rössle-Cancer Clinics aimed to establish xenografts from colorectal carcinomas. Starting with 35 surgical samples, we were able to establish 15 transplantable lines in nude mice. The xenografts coincided with the original tumor both in histology and in the expression of tumour-associated markers

(EpCAM, E-cadherin, CEA). Their response rate towards clinically used drugs was 33% for 5-Fluorouracil, 100% for Irinotecan, and 57% for Oxaliplatin. In five patients who received chemotherapy for synchronous metastases, the responsiveness of the malignancy correlated excellently with the responsiveness of the corresponding xenografts. All of the xenografts expressed the proliferation marker Ki67 and the nuclear enzyme Topoisomerase II. Sixty-four percent (64%) of the xenografts expressed the mutated tumour suppressor p53. Interestingly, the detection of *K-ras* mutations in codon 12 in 30% of the carcinomas coincided with a low response to Oxaliplatin.

These well-characterized models are useful tools for the pre-clinical development of novel therapeutic approaches and for investigating translational research aspects.

Procedure for in vivo gene transfer

The clinical use of gene therapeutic strategies for the treatment of cancer is still hampered by insufficient transfer efficiencies and specificities in the in vivo situation. We investigated a non-viral gene transfection system utilizing lipoplexes. As an additional component, alkyl phospholipids (APL) were included because of their known interference with cellular membranes. The APL lipoplexes showed in vitro the highest transfer efficiencies into colon carcinoma cells. The LacZ reporter gene expression in colon xenografts was equally intense as revealed after use of lipofectin. A suicide

A Nude mouse bearing a patient-derived colon carcinoma xenograft
B NOD/SCID mice xenotransplanted with a human leukemia



therapy with the cytosine deaminase gene + 5-Fluorocytosine in a murine colon carcinoma resulted in the highest tumor growth inhibition when APL containing lipoplexes were used for gene transfer.

Stem cell engraftment and proliferation

Continuing former studies, we were further interested in methods for the controlling of the engraftment potential of haematopoietic stem cells. For this purpose, cord blood-derived CD34-positive progenitor cells were transplanted into highly immunodeficient NOD/SCID mice. In order to subsequently monitor a successful engraftment, we developed a sensitive PCR method for the detection and real-time quantification of human cells in xenotransplantation systems. This universally applicable method targets a 850-bp fragment of the alpha-satellite DNA on human chromosome 17. The method allows for the detection of one human cell in 10^6 mouse cells and could monitor the engraftment rate of stem cells in a time dependent fashion.

A further study explored the influence of human interleukin-3 (IL-3) on the production of human cordblood derived haematopoietic cells in NOD/SCID mice. The background for that study was the knowledge that not all haematopoietic cytokines are cross-reactive between mice and human. In particular, IL-3 is highly species-specific but plays a crucial role in hematopoiesis. We used, as a source for human IL-3, a stably transfected rat fibroblast cell line, which was co-transplanted to NOD/SCID mice. Rat-IL-3 mice displayed a higher engraftment of human haematopoietic cells in bone marrow, spleen, and peripheral blood when compared with mice bearing a mock-cotransplant. Successive experiments with long term bone marrow cultures or secondary transplantations showed that IL-3 could also play a role in the depletion of haematopoietic stem cells from the chimeric bone marrow of mice.

Ongoing studies with cordblood stem cells investigate the differential expression of adhesion and apoptosis related markers after co-cultivation with tissue specific factors. These experiments are the precondition for scheduled *in vivo* studies concerning the transdifferentiation potential of early haematopoietic progenitors.

Selected Publications

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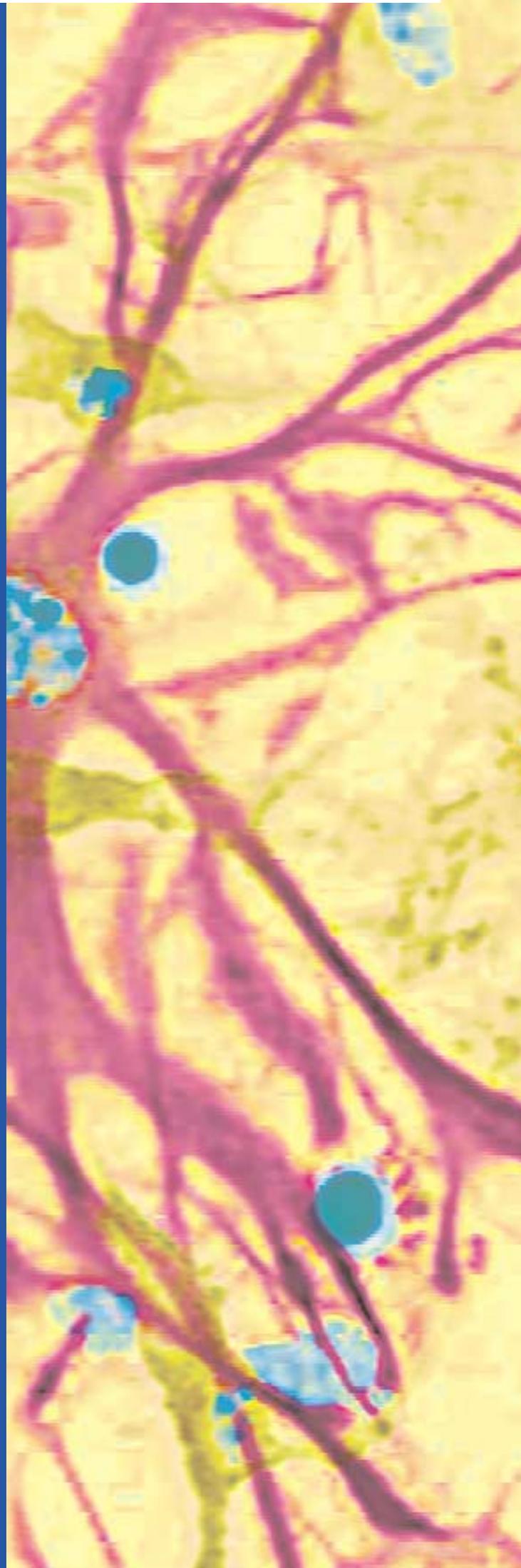
Function and Dysfunction of the Nervous System

Signalling Pathways in the
Nervous System

Pathophysiological Mechanisms
of Neurological and Psychiatric
Disorders

Imaging of the Live Brain

Coordinators:
Carmen Birchmeier
Helmut Kettenmann



Function and Dysfunction of the Nervous System

Carmen Birchmeier
Helmut Kettenmann

A single neuron can contact many neuronal and glial cells. The structure and function of the nervous system are thus very complex. The understanding of the molecular mechanisms used to establish and maintain neuronal circuits, or to process and store information in such circuitry, is still limited. Recent advances in methodology and the available genome sequence of humans and model organisms now allow a more systematic molecular analysis and will accelerate the furthering of our knowledge.

The research program in Neuroscience at the MDC focuses on molecular and cellular aspects and relies on the use of molecular biology, biochemistry, genetics, immunocytochemistry, electrophysiology, and anatomy to understand the formation and function of the nervous system. Particular emphasis is also devoted to the analysis of mechanisms that underlie neuropathological conditions.

Several of the recent findings of the department have important medical implications. Erich Wanker and his group study the aggregation of proteins as they occur in Alzheimer's, Parkinson's, or Huntington's disease. Agents that prevent these aggregates, also known as amyloid plaques, are expected to be beneficial for the treatment of these diseases. Using automated cell-based and cell-free screening assays, they recently identified such inhibitors and are currently testing their activity in animal models, i.e. transgenic flies and mice. Compounds able to reduce aggregate formation and neurological symptoms *in vivo* are candidate substances for clinical trials for these neurodegenerative diseases (Ross et al., Proc. Natl. Acad. Sci. USA 100, 1-3, 2002).

Stimulation of neuronal regeneration in patients with neurodegenerative disease might be an interesting therapeutic tool for the future. Gerd Kempermann and Alistair Garratt aim to understand the properties and behaviour of neuronal stem cells. Gerd Kempermann and his team could recently distinguish stem cell populations in the adult hippocampus which respond differently to physical activity or changes in environmental stimulation (Kronenberg et al., J. Comp. Neurol. 467,

Funktion und Dysfunktion des Nervensystems

Carmen Birchmeier
Helmut Kettenmann

Ein einzelnes Neuron kann viele Neurone und Gliazellen kontaktieren, Struktur und Funktion des Nervensystems sind daher sehr komplex. Wir verstehen bisher nur begrenzt die molekularen Mechanismen, die die neuronalen Schaltkreise aufbauen und erhalten. Ebenso ist noch nicht bekannt, wie Informationen in solchen Netzwerken verarbeitet und gespeichert werden. Technologische Fortschritte und die Verfügbarkeit der Genomsequenz von Menschen wie Modellorganismen erlauben nun eine systematischere Analyse und werden uns weitere Einblicke in die Funktionen des Gehirns ermöglichen.

Das Forschungsprogramm Neurowissenschaften am MDC stellt die molekularen und zellulären Aspekte in den Vordergrund. Es nutzt Ansätze der Molekularbiologie, Biochemie, Genetik, Immunzytochemie, Elektrophysiologie und Anatomie, um die Entwicklung und Funktion des Nervensystems zu verstehen. Ein besonderer Schwerpunkt liegt auf der Analyse der Mechanismen von neuropathologischen Veränderungen.

Eine Anzahl neuer Ergebnisse aus dem Bereich hat wichtige medizinische Implikationen. Erich Wanker und seine Gruppe untersuchen die Aggregation von Proteinen, die eine wichtige Rolle bei Alzheimer, Parkinson und Huntington Erkrankungen spielen. Substanzen, die eine solche Aggregation verhindern, können den Verlauf dieser Erkrankungen positiv beeinflussen. In einem automatisierten Screening-Verfahren haben sie kürzlich solche Inhibitoren entdeckt und testen jetzt deren Aktivität in Tiermodellen, wie zum Beispiel transgenen Fliegen oder Mäusen. Substanzen, die die Aggregatbildung reduzieren und neurologische Symptome lindern, sind potentielle Substanzen für klinische Studien (Ross et al., Proc. Natl. Acad. Sci. USA 100, 1-3, 2002).

Die Stimulation neuronaler Regeneration bei Patienten mit neurodegenerativen Erkrankungen könnte ein interessantes therapeutisches Werkzeug der Zukunft sein. Gerd Kempermann und Alistair Garratt studieren die Eigenschaften und das Verhalten neuronaler Stammzellen. Gerd Kempermann und sein Team konnten kürzlich drei Populationen im

455-463, 2003). Alistair Garratt is analysing the function of growth factors in the maintenance and differentiation of adult neuronal stem cells. Cortical spreading depression is a slowly propagating wave of neuronal depolarization in the mammalian brain, and is thought to play a role in pathological conditions, for instance in migraine headaches.

Neuronal depolarization was found to be accompanied by Ca^{2+} activity of glia, the cell type studied by Helmut Kettenmann's group. Waves of Ca^{2+} elevations spread within a glial population of the brain, the astrocytes. Interestingly, the astrocyte Ca^{2+} wave penetrates a larger territory than the activity of neurones and by this represents a self-reliant phenomenon (Peters et al., *J. Neurosci.* 23, 9888-9896, 2003).

Christiane Alexander found that mutations in the OPA-1 gene result in degeneration of retinal ganglion cells. Patients affected by such a gene defect face a loss of vision starting within the first two decades of their life. Her research focuses on the functional role of this protein, which is distributed ubiquitously, but its malfunction leads to a primary retinal defect.

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 central nervous system by projecting 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. Gary Lewin's group used microarray techniques to identify a calcium channel that is specifically expressed in sensory neurons that detect light brush. This channel enhances the sensitivity of mechanoreceptive neurons and its expression is a unique marker to define this subtype of sensory neuron (Shin et al., *Nature Neuroscience* 6, 724-730, 2003).

How neurons form precise and selective connections is a central question in developmental neurobiology. The growth cone of sensory neurons detects guidance cues that are necessary for the innervation of the spinal cord. The cGMP messenger pathway can convert repulsive pathfinding cues to attractive ones, and is therefore an important modulator of neuronal pathfinding. Fritz Rathjen and his collaborators characterized the significance of cGMP signalling in sensory innervation of the spinal cord. In mice lacking the gene encoding the cGMP-dependent protein kinase I, sensory axons fail to bifurcate correctly at the entrance zone of the spinal cord. Instead they grow directly to the central canal in these mutant mice. As a result, the animals have lost their reflex response to pain (Schmidt et al., *J Cell Biol.* 159, 489-498, 2002). Dorsal horn neurons are known to be diverse, but nevertheless develop from only a few distinct postmitotic neuron types, which were recently defined.

erwachsenen Hippocampus unterscheiden, die unterschiedlich auf physische Aktivität oder Veränderung von Umweltreizen reagieren (Kronenberg et al., *J. Comp. Neurol.* 467, 455-463, 2003). Alistair Garratt untersucht die Funktionen von Wachstumsfaktoren, die erwachsene neuronale Stammzellen stabilisieren und deren Differenzierung steuern. „Cortical spreading depression“ ist eine sich langsam ausbreitende Welle neuronaler Hemmungen im menschlichen Gehirn, und man vermutet, dass sie bei neurologischen Symptomen eine Rolle spielen wie zum Beispiel bei Migräne.

Diese neuronale Depolarisation ist begleitet von einer Kalziumaktivität der Gliazellen, die in Helmut Kettenmanns Labor untersucht werden. Diese Kalziumerhöhungen breiten sich wellenartig in einer glialen Zellpopulation des Gehirns, den Astrozyten, aus. Interessanterweise breitet sich die Kalziumwelle der Astrozyten über einen größeren Bereich des Gehirns aus als die Aktivität der Neurone (Peters et al., *J. Neurosci.* 23, 9888-9896, 2003).

Christiane Alexander konnte zeigen, dass Mutationen im OPA-1 Gen zu einer Degeneration der retinalen Ganglienzellen führt. Patienten mit einem solchen Gendefekt verlieren ihr Augenlicht innerhalb der ersten beiden Lebensjahrzehnte. Sie untersucht daher die funktionelle Bedeutung des Proteins, das zwar in allen Zellen verbreitet ist, dessen Fehlfunktion jedoch primär die Retina betrifft.

Der Tastsinn ist nicht eine einzelne Wahrnehmung. Neurowissenschaftler unterscheiden die Wahrnehmung von Textur, Temperatur und Schmerz. Definierte Neurone in den sensorischen Ganglien innervieren die Haut und leiten ihre Information an das Zentralnervensystem weiter, indem sie ins Rückenmark projizieren. Die Neurone im Rückenmark integrieren und verarbeiten diese sensorische Information und leiten sie in höhere Hirnzentren weiter. Sensorische Neuronen, die leichte Berührung der Haut erkennen, unterscheiden sich von denen, die schmerzvolle Hitzeerize erkennen. Bisher konnten sie genetisch nicht unterschieden werden. Gary Lewins Gruppe nutzte die Technik der Micro-Arrays, um einen Kalziumkanal zu finden, der nur in sensorischen Neuronen exprimiert wird, die leichte Berührung erkennen. Dieser Kanal verstärkt die Sensitivität der mechanosensitiven Neuronen, und durch seine Expression kann dieser Subtyp von sensorischen Neuronen erkannt werden (Shin et al., *Nature Neuroscience* 6, 724-730, 2003).

Wie Neurone präzise und selektive Verbindungen aufbauen, ist eine zentrale Frage in der Entwicklungsneurobiologie. Der Wachstumskegel sensorischer Neurone erkennt Wegmerkmale, die notwendig sind, um das Rückenmark zu innervieren. Der cGMP Signalweg kann inhibitorische Signale in positive verwandeln und ist daher ein wichtiger Modulator der neuronalen Zielfindung. Fritz Rathjen und seine Mitarbeiter untersuchen die Bedeutung des cGMP Signalweges für die sensorische Innervation des Rückenmarks. In Mäusen, bei denen das Gen für die cGMP-abhängige Proteinkinase 1 ausgeschaltet ist, verzweigen sich die sensorischen Axone nicht mehr korrekt in der Eingangszone des Rückenmarks. Stattdessen wachsen sie in diesen Mutanten direkt zum zentralen Kanal. Dies hat zur Folge, dass die Tiere eine verminderte Reflexantwort für Schmerz zeigen (Schmidt et al., *J Cell*

Carmen Birchmeier and her group identified an important gene product, the homeodomain factor Lbx1, which distinguishes two major neuronal classes in the dorsal spinal cord and is an important determinant of their distinct differentiation programs. In Lbx1 mutant mice, highly abnormal differentiation of dorsal horn neurons is accompanied by alterations in the innervation of the dorsal horn by cutaneous sensory neurons. These changes in circuitry are accompanied by deficits in sensory reflexes (Müller et al., *Neuron* 34, 551-562, 2002). Stefan Britsch studies the genome-wide expression of genes during spinal cord development, and currently analyses the function of homeodomain factor Gbx1 in the development of spinal cord neurons.

The Neuroscience research program has expanded considerably during the reported period. The research groups of Erich Wanker and Carmen Birchmeier joined the department, and Gary Lewin, previously a junior group leader in the department, received tenure and is now full professor at the Humboldt University of Berlin. Since the completion of the communication centre of the MDC, larger scientific conferences can be accommodated on the campus. One neuroscience conference that took place on the campus was the VI European Meeting on Glial Cell Function in Health and Disease in September 2003. It was attended by more than 500 scientists from all over the world.

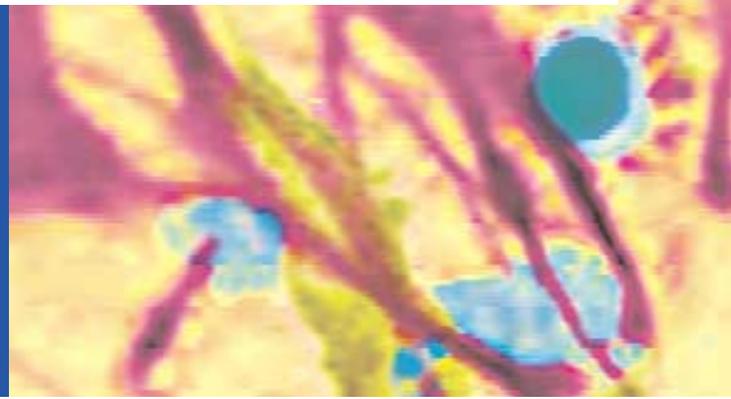
Other activities were a meeting of the faculty and students of the Neuroscience Department in 2003 at Chorin in Brandenburg, which was used to present and to discuss ongoing research and foster interactions in a relaxed and informal environment. This department meeting alternates with the Berlin Neuroscience Forum that is attended by neuroscientists from all the neuroscience institutes in Berlin, and that took place in Liebenwalde in 2002. A seminar series that is organized jointly by all neuroscience faculty members was established in 2003. It developed into a very successful lecture series that is regularly attended by many scientists and students, both from the neuroscience as well as other MDC departments.

Biol. 159, 489-498, 2002). Neurone des dorsalen Horns setzen sich bekannterweise aus verschiedenen Populationen zusammen. Sie entwickeln sich jedoch, wie kürzlich gezeigt, aus wenigen Typen von postmitotischen Neuronen.

Carmen Birchmeier und ihre Gruppe identifizierten ein wichtiges Genprodukt, den Homeodomänfaktor Lbx1, durch dessen Expression sich zwei wichtige neuronale Populationen im dorsalen Rückenmark unterscheiden und der ein wichtiger Faktor für ihr Differenzierungsprogramm ist. In den Lbx mutanten Mäusen entwickeln sich die Neurone des dorsalen Horns abnormal, und dies ist begleitet von einer veränderten Innervation des dorsalen Horns durch die sensorischen Neurone der Haut. Diese veränderten neuronalen Verbindungen gehen mit defekten sensorischen Reflexen einher (Müller et al., *Neuron* 34, 551-562, 2002). Stefan Britsch untersucht die Genomweite Expression von Genen während der Rückenmarksentwicklung und studiert derzeit die Bedeutung des Homeodomänfaktors Gbx1 während der Entwicklung der Rückenmarksneurone.

Im Berichtszeitraum wurde das Forschungsprogramm Neurowissenschaften stark erweitert. Die Forschungsgruppen von Erich Wanker und Carmen Birchmeier sind zu dem Koordinationsbereich gestoßen, und Gary Lewin, zuvor Nachwuchsgruppenleiter im Bereich, leitet nun eine etablierte Gruppe und wurde zum Professor an der Humboldt Universität zu Berlin ernannt. Seit Eröffnung des Kommunikationszentrums am MDC ist es möglich, große Konferenzen am Standort durchzuführen. Ein solches Ereignis war das „6th European Meeting on Glial Cell Function in Health and Disease“ im September 2003, das mehr als 500 Wissenschaftler aus aller Welt anzog.

Zu den weiteren Aktivitäten gehörte ein gemeinsames Symposium aller Neurowissenschaftler des MDC, das 2003 in Chorin, Region Brandenburg, durchgeführt wurde und als Forum für die Diskussion neuester wissenschaftlicher Ergebnisse und zur Planung weiterer Zusammenarbeiten genutzt wurde. Dieses Department-Treffen wechselt sich mit dem Berlin Neuroscience Forum ab, bei dem Neurowissenschaftler aus allen Berliner Institutionen zusammenkommen. Der Koordinationsbereich etablierte im Jahr 2003 ein gemeinsames Vortragsprogramm, das für viele Wissenschaftler und Studenten sowohl im Neuro- als auch aus anderen Bereichen des MDC attraktiv ist.



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

Carmen Birchmeier

We are using mice as a model organism for the functional analysis of genes important for embryonic development and disease. 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, 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 by the use of mouse genetics.

The role of Lbx1 in spinal cord development

Thomas Müller, Henning Brohmann

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. Prior to the emergence of postmitotic neurons, neuronal progenitor cells possess regional identities and distinct progenitor populations generate the different neuronal subtypes.

In the dorsal portion of the spinal cord, progenitor cells are patterned by extrinsic signals. Members of the BMP and wnt families are expressed in or near the roof plate at the time dorsal progenitor domains are established. Six postmitotic neuron types (named dorsal interneurons (dI) and numbered from 1 to 6 along the dorso-ventral axis) can be distinguished. We showed that these six neuron types can be classified based on the expression of the Lbx1 gene (Fig. 1): Class A neurons (dI1-dI3) do not express Lbx1, arise in the dorsal portion of the dorsal spinal cord, require dorsal signals for specification, and settle in the deep dorsal horn. In contrast, class B neurons (dI4-dI6) express Lbx1, emerge independently from roof plate signals in the ventral portion of the dorsal spinal cord, and most eventually settle in the ventral spinal cord and in the deep dorsal horn.

During a later phase known as the second neurogenic wave (E12.5 in the mouse), most neurons born in the dorsal spinal cord express Lbx1 and, thus, have a class B character. We defined two late Lbx1⁺ neuronal subtypes (dIL^A and dIL^B) (Fig. 2) that arise in a salt-and-pepper pattern and settle in the dorsal horn. Lbx1 not only delineates two major dorsal neuronal classes but also specifies the differentiation program of class B neurons. In Lbx1 mutant mice, early and late class B neurons express transcription factors typical of class A neurons. Conversely, misexpression of Lbx1 in the chick spinal cord suppresses the emergence of class A neurons and, instead, induces the generation of class B neurons (Fig. 3). The abnormal differentiation of the dorsal horn neurons in Lbx1 mutants is accompanied by changes in the histology and circuitry of the spinal cord. Thus, Lbx1 is essential for the specification and differentiation of class B neurons and suppresses the development of class A neurons (Müller et al, 2002).

We are systematically extending these studies and are analyzing the function of other genes in dorsal horn development.

The Neuregulin/ErbB signaling system

Alistair Garratt, Thomas Müller, Cemil Özcelik, Simone Lier

Neuregulin-1 is an EGF-like growth and differentiation factor that signals through tyrosine kinase receptors of the ErbB family. Two receptors, ErbB3 and ErbB4, bind Neuregulin-1

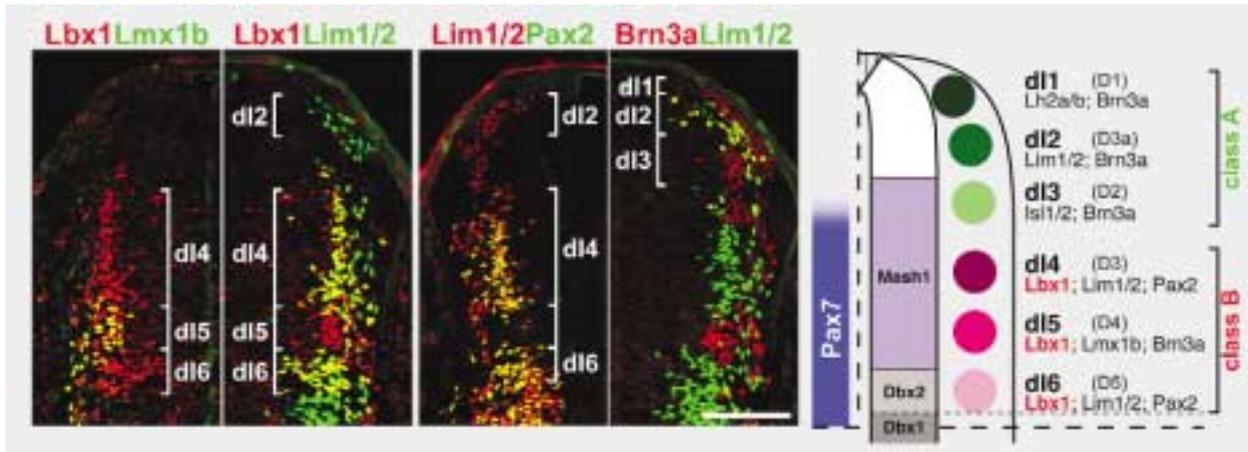


Fig. 1. Lbx1 marks class B neurons in the dorsal spinal cord at E10.5. Sections of E10.5 spinal cords were stained with antibodies against the markers shown above the panels (A-D). Data are summarized in E. Interneuron types dl1-6 are marked. Bar: 100 μ m.

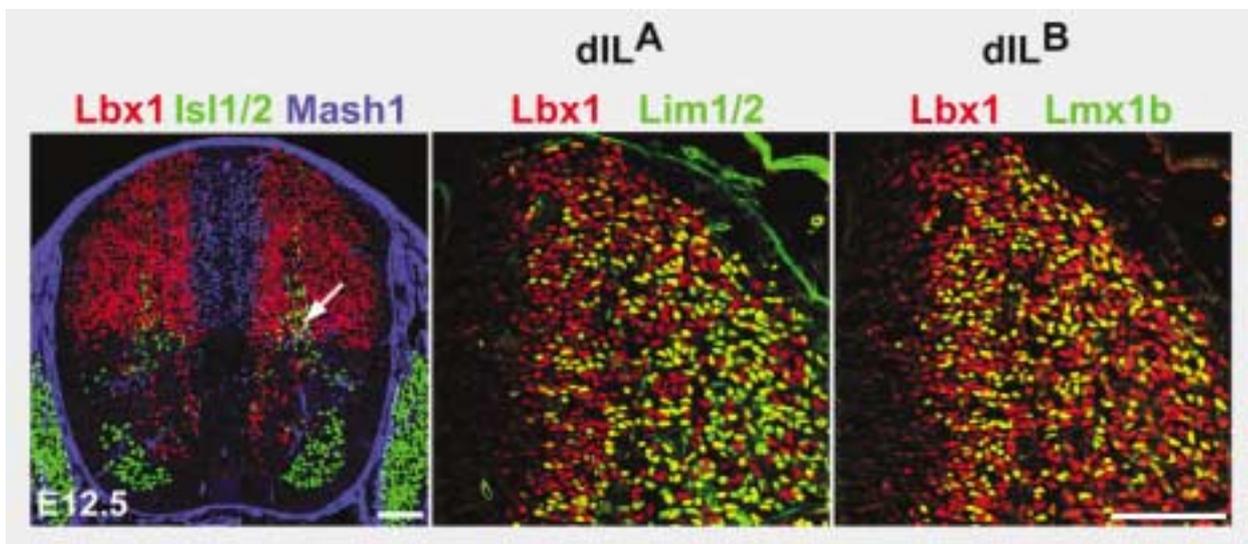


Fig. 2. The late class B neuron types dILA and dILB arise from the entire Mash1+ progenitor domain in a salt-and-pepper pattern at E12.5. Sections of E12.5 spinal cords were stained with antibodies as shown above the panels. Isl1/2+ dl3 neurons have reached already their final location in the deep dorsal horn (arrow, left panel). Lbx1+ neurons arise from the entire Mash1+ progenitor domain and settle in upper layers of the dorsal horn. dILA and dILB neurons arise in a salt-and-pepper from the Mash1+ progenitor domain (middle and right panels). Bars: 100 μ m.

with high affinity and are expressed in distinct patterns during development: ErbB3 is expressed in neural crest and glial cells, whereas ErbB4 is expressed in the heart. A third receptor, ErbB2, is present ubiquitously and acts as an essential co-receptor. Accordingly, functional receptor complexes *in vivo* are either ErbB2/3 or ErbB2/4 heteromers, depending on the organ or cell type. We have introduced null-mutations into mouse Neuregulin-1, ErbB2 and ErbB3 genes. All of these mutations cause either embryonic or postnatal lethality. Therefore, we introduced more subtle mutations that eliminate particular isoforms of Neuregulin, or conditional mutations in the ErbB2 receptor. Together, the analysis of these mutants revealed diverse, essential functions of the Neuregulin/ErbB signaling system in the development of the heart, the neural crest cells, and the peripheral nervous system.

Role of the Neuregulin signaling system in heart development and function

Alistair Garratt, Cemil Özcelik

Mice with null-mutations in Neuregulin/ErbB2 display deficits in heart development, that causes embryonic lethality at mid-gestation. This genetic analysis revealed for the first time a role of Neuregulin/ErbB2 in cardiomyocytes which is also of interest for subsequent observations of side-effects of ErbB2 antibody therapy used now in patients for tumor treatment (a proportion of such patients develops cardiomyopathies). To analyze whether ErbB2 has an essential role in adult heart function, we used the Cre-loxP technology to mutate ErbB2 specifically in cardiomyocytes. Such conditional mutant mice develop a severe dilated cardiomyopathy with signs of cardiac dysfunction appearing by the second postnatal

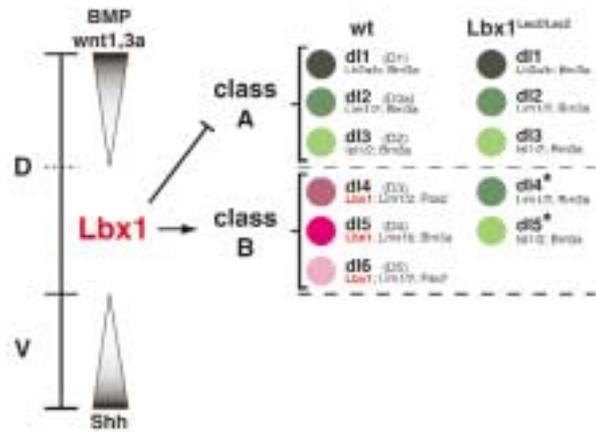


Fig. 3. Schematic diagram of dorsal spinal cord neurons and of the function of Lbx1 in their development. BMP and Shh, secreted factors that pattern the dorsal (D) and ventral (V) progenitor domains, are indicated at the left. The class A (green) and B (red) neuronal subtypes generated in the dorsal spinal cord are indicated. The positions along the dorso-ventral axis at which these neurons arise are shown, as well as the homeodomain factor code they express in wild-type mice. Lbx1 suppresses the emergence of class A neurons, and specifies the development of class B neurons (indicated). In Lbx1^{LacZ/LacZ} mice, class B neurons assume abnormal molecular characteristics (d4⁺ and d5⁺ neurons), and express the indicated homeodomain factor combinations typical of class A neurons (shown on the right).

month. We infer that signaling from the ErbB2 receptor, which is enriched in T-tubules in cardiomyocytes, is crucial not only during heart development but also for the correct functioning of the adult heart. Conditional ErbB2 mutants provide a novel animal model of dilated cardiomyopathy and will allow for a rigorous assessment of the adverse effects of anti-ErbB2 antibodies on cardiac function.

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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.

During development, distinct cell types are generated along the dorso-ventral axis of the neural tube. Neurons that process and relay sensory information primarily reside in the dorsal portion of the spinal cord, whereas neurons that integrate and direct motor control are located ventrally. This regionally restricted generation of different neuron types, and subsequent establishment of complex neuronal circuits, are governed by extracellular signals and transcriptional networks. In order to identify novel genes involved in control of these processes, we have used global gene expression analyses with high-density oligonucleotide microarrays.

We have detected several novel or only partially characterized genes with unknown functions in the development of the dorsal spinal cord. Expression analysis has revealed that many of

these candidate genes are initially expressed in late born, postmitotic dorsal neurons. Subsequently, expression becomes restricted to distinct neuronal subpopulations within the superficial layers of dorsal horn (see figure). Neurons located within these layers are known to be involved in the processing and relaying of nociceptive and mechanosensory information. Thus, our preliminary data indicate that our candidate genes are potentially involved in the specification, terminal differentiation, and/or positioning of specific neuronal subpopulations of the superficial dorsal horn. Current work of the group focuses on the functional characterization of these genes by targeted deletion in mice.

Selected Publications

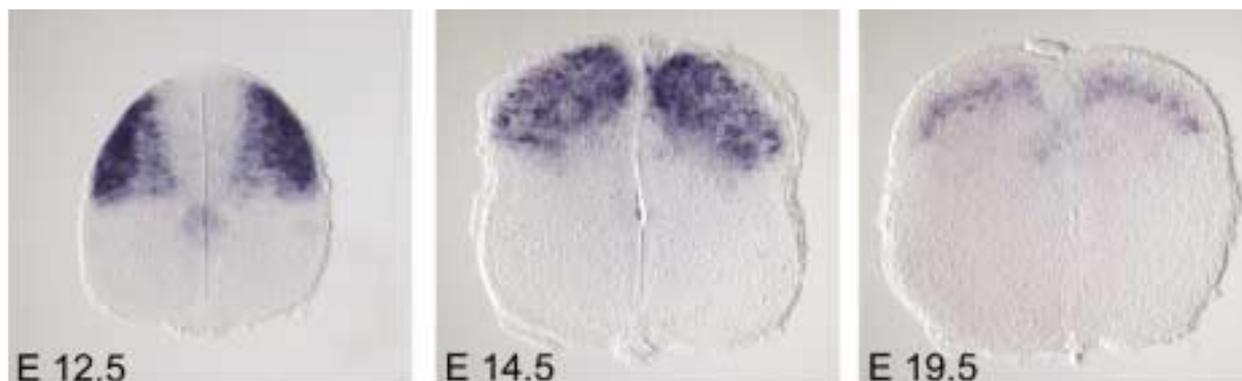
Özcelik C, Erdmann B, Pilz B, Wettschurek N, Britsch S, Hübner N, Chien KR, Birchmeier C, Garratt AN (2002) Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. *PNAS* 99: 8880-8885.

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Spatio-temporal expression of the homeodomain transcription factor Gbx1 during embryonic development of the spinal cord. Gbx1 was identified with Affymetrix microarrays and predicted to be differentially expressed in the dorsal spinal cord. Shown are cross-sections through the spinal cord of wildtype mice at different developmental stages (E12.5 – E19.5). Gene expression is visualized by in situ hybridization.



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Genetic Analysis of Nervous System Development and Function

Alistair N. Garratt (Helmholtz Fellow)

The development and homeostasis of multicellular organisms depends on the ability of cells to receive extracellular signals and to transmit this information into the cell interior. Various types of cellular response may ensue, for example cells may alter their migratory behaviour, change their proliferation rate, progress into a final differentiated state, or undergo apoptosis. We are interested in signalling systems required for development and functioning of the mammalian peripheral and central nervous systems. We employ classical knock-out and conditional gene targeting strategies in order to address the roles of signalling molecules *in vivo* in the mouse. One avenue of particular medical interest is the role of growth factor signalling in the maintenance of neuronal stem cells and/or their progeny in the adult.

Conditional mutation of the receptor tyrosine kinase ErbB2 in the nervous system

The proto-oncogene ErbB2 (c-neu or HER2 in human) encodes a receptor tyrosine kinase, which is frequently over-expressed in human tumours and is the target of an antibody-based therapy for metastatic mammary carcinoma (trastuzumab/Herceptin). ErbB2 functions as a co-receptor for other members of the ErbB family of receptor tyrosine kinases. In particular, the EGF-like growth factor Neuregulin-1 transmits signals via ErbB2/ErbB3 and ErbB2/ErbB4 heteromers. The ErbB/Neuregulin-1 signaling system is essential for normal embryonic development and continues to function in various tissues and organs in the adult animal, including the central nervous system. Previously, working together with Prof. C. Birchmeier (MDC), we employed conditional gene targeting (cre-loxP technology) to inactivate the receptor tyrosine kinase ErbB2 in myelinating Schwann cells and cardiomyocytes. We are currently focusing on analysis of mice in which ErbB2 has been mutated in the central nervous system, using a Brn4-cre transgene. These studies indicate that ErbB signaling is important for the maintenance of specific groups of neurons in the adult nervous system.

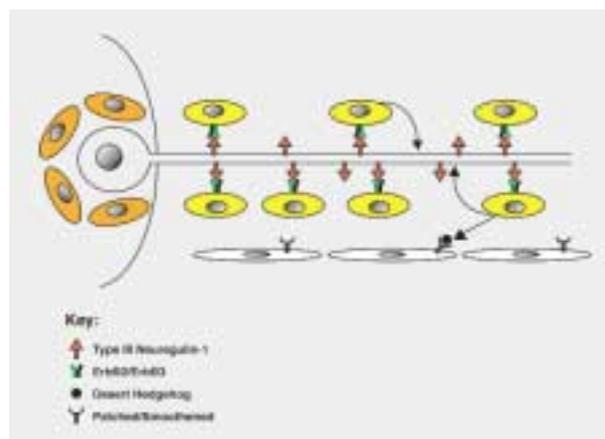
Characterization of novel molecular components involved in pain perception

The perception of acute pain stimuli is crucial in alerting an organism to environmental dangers, and is thus an important survival mechanism. In contrast, chronic pain states in patients are debilitating conditions, generally refractory to current treatments, and lead to substantial losses in quality of life. Currently, considerable effort is being invested worldwide to better characterize the molecular bases of pain circuitry and enable the design of novel therapies for acute and chronic pain.

One area of particular importance for the reception of pain modalities is the superficial dorsal horn of the spinal cord, in particular, the *substantia gelatinosa*. This region is innervated principally by non-myelinated projections from pain-sensing peripheral neurons (nociceptors), and can be identified as a semi-translucent region in the head of the dorsal horn. Despite the significance of this area to pain processing, the molecular characteristics of the maturing and adult dorsal horn are as yet poorly defined.

We used an Affymetrix microarray-based screen to identify genes with enriched expression in the superficial dorsal horn of the adult mouse spinal cord. These could be grouped into various classes, including genes encoding neuropeptides, neuropeptide and glutamate receptors, cation channels, transcription factors, and calcium-binding proteins. Expression patterns of selected genes were verified by *in situ* hybridization. These include c-kit, encoding a receptor tyrosine kinase with multiple developmental functions, which is also known to be expressed by nociceptive neurons, and the three mouse homologues of the *Drosophila* gene *teashirt* (*tsh*), a homeotic gene which encodes a zinc-finger protein involved in trunk identity in the fly. All four genes are widely expressed during mouse development, and have interesting expression patterns in both the peripheral and central nervous systems.

During the development of the peripheral nervous system, neurons and their axons provide signals that control development of satellite glia (orange) and Schwann cells (yellow), respectively. One axonally derived signal that controls the numbers of early Schwann cell precursors, and which also regulates the thickness of the insulating myelin sheath made by mature Schwann cells, is provided by type III Neuregulin-1 (red arrows). This growth factor activates the ErbB2/ErbB3 receptor heteromer (black and green) expressed by cells of the Schwann cell lineage. Schwann cells in turn provide other signals (black arrows, circle) important for the maintenance of neurons and axons, and for the development of the perineurium (white ellipsoids).



We are now generating knock-out and conditional alleles of the three mouse teashirt genes and have obtained a mouse strain carrying a null mutation in the *c-kit* gene from the Jackson laboratories. We will analyse the function of these genes in development and maintenance of the nervous system. Furthermore, as *c-kit* mutants can be generated as viable homozygotes, we can begin to investigate the phenotype of the pain-sensing circuitry of adult mouse mutants directly using electrophysiological techniques.

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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. While in the last years the group has studied aspects related to all three types of glial cells, 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 detect neuronal activity?

In the last years, we have learned that astrocytes in cell culture have the capacity to express almost all receptors known to mediate synaptic transmission. One of our best-studied examples is the Bergmann glial cell in the cerebellum, a morphologically specialized astrocyte. We found that activity of parallel fibres, the axons of the granule cells synapsing onto Purkinje neurons, triggers a calcium signal in Bergmann glial cells. With moderate activity, the signal can be confined to a sub region of the cell. This sub region has a morphological correlate, the microdomain. With more intense stimulation the signal spreads to the soma. This form of

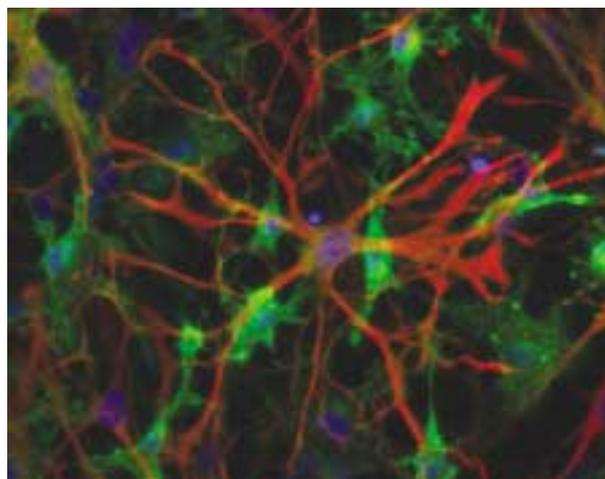
neuron-glia interaction is mediated by nitric oxide (NO) which is known to be released from parallel fibres. We speculate that these units could feedback information on a defined population of synapses, namely those which are enwrapped by a given microdomain.

In a collaborative grant with the Bogomoletz Institute of Physiology, Kiev, we have obtained evidence that astrocytes receive synaptic input. A transgenic animal model developed in our laboratory made it possible to identify small astrocyte compartments on the ultrastructural level.

2. How do astrocytes communicate among each other?

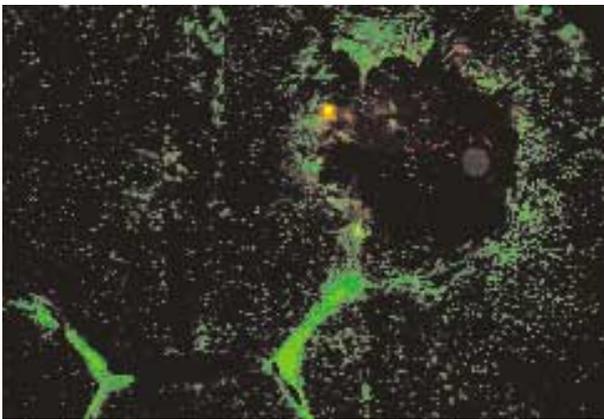
From experiments in cell culture and from studies in the isolated retina, it has become evident that astrocytes can communicate over large distances (<0.5 mm) via calcium signalling in the form of waves. We have found conditions to elicit and record such Ca^{2+} waves in slices containing the corpus 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. The wave travels with a low speed of about 10 $\mu\text{m/s}$ similar as in culture and is thus 1,000,000 times slower than the neuronal action potential. In brain tissue, the calcium wave is not restricted to astrocytes, but also activates cells of the oligodendrocyte lineage and the microglial cells. Using cortex as a model of grey matter, we found that neuronal activity can trigger an additional Ca^{2+} activity in astrocytes which spreads faster than the intrinsic astrocyte wave. This neuron-driven astrocyte activity is, for instance, observed during cortical spreading depression, a phenomenon associated with the aura of migraine.

Rat-Astrocytes (red) and microglia (green) in primary culture
(red: staining against GFAP
green: ILB4-staining)





The figure displays the extent of biocytin coupling of a single astrocyte in cortex. Note that astrocyte endfeet delineate a blood vessel.



In an experimental tumor model in mouse, glioma cells (labelled red) attract neural precursor cells (labelled green)

3. 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 GABA_B receptors suppressed indicators of microglial activation such as the release of IL-6. A similar reduction in proinflammatory mediators was found with activation of purinergic receptors which are important signalling molecules for astrocyte activity. These findings support the hypothesis that microglial cells are less prone to activation when they sense normal neural activity.

4. What factors control activation of microglial cells?

Microglial cells are activated by any type of brain injury or pathologic disturbance. Using bacterial lipopolysaccharide (LPS) as a tool to activate cultured mouse microglia, we studied alterations in the intracellular calcium concentration ($[Ca^{2+}]_i$) and in the receptor-evoked generation of transient calcium signals. LPS treatment led to a chronic elevation of basal $[Ca^{2+}]_i$ along with a suppression of evoked calcium signalling. Our findings suggest that chronic elevation of basal $[Ca^{2+}]_i$ attenuates receptor-triggered calcium signalling. Moreover, increased $[Ca^{2+}]_i$ is required, but by itself not sufficient, for release of NO and certain cyto/chemokines. Elevation of basal $[Ca^{2+}]_i$ could thus prove to be a central element in the regulation of executive functions in activated microglia.

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

A candidate for signalling neuronal injury to microglial cells is the 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 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.

6. What are the physiological properties of gliomas and how do they compare to normal glia?

The majority of tumors of the central nervous system 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 address the question whether microglial cells influence tumor cell behaviour. In a long-term 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.

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Brain Energy Metabolism

Susanne Arnold (Emmy Noether Research Group)

Our group investigates astrocytes, the most prominent cell group in the mammalian brain, and the regulation of their energy metabolism in coordination with neuronal activity under physiological and pathological conditions. Astrocytes, strategically well positioned between capillaries and neurons, can regulate energy substrate availability, such as glucose, and energy production in response to neuronal energy consumption.

We apply various fluorophores and protein sensor molecules to register changes in cytosolic ion and second messenger concentrations in astrocytes upon neuronal activation, correlating them with changes of parameters of the energy metabolism, such as mitochondrial membrane potential, NAD(P)H content, and mitochondrial calcium. So far, we could show that as a consequence of increases in astrocytic cytosolic calcium, the mitochondrial membrane potential transiently depolarizes and the NAD(P)H content decreases. The underlying mechanisms are not well understood yet and will be studied in detail.

Glucose is the major energy substrate in the brain, which is mainly taken up by astrocytes and converted into lactate to fulfill neuronal energy requirements. Taking into account the importance of glucose for the energetic balance of brain cells, we studied the impact of hypoglycemia on astrocytic calcium levels and signaling. We found that hypoglycemia induces an increase in cytosolic calcium and an impairment of neurotransmitter-triggered calcium signaling in astrocytes. The hormone, 17β -estradiol, protects astrocytes from the impact of hypoglycemia on resting Ca^{2+} levels and on neurotransmitter-triggered Ca^{2+} signaling. The mechanism of the hormone action is a focus of our further studies.

Cytochrome c oxidase (COX), the terminal and rate-limiting enzyme of the mitochondrial respiratory chain, is engaged in oxidative energy metabolism. The mammalian enzyme (see figure) is composed of 3 catalytic, mitochondrial-encoded and 10 regulatory, nuclear-encoded subunits. In mammals, isoforms have been identified for subunits IV, VIa, VIb, VIIa and VIII, which are expressed in a tissue specific and deve-

lopmental manner. In previous studies, we found that the catalytic activity of COX is regulated according to the energy level of the cell: binding of ATP, the indirect product of the COX reaction, to subunit IV of COX at high ATP/ADP ratios leads to an allosteric inhibition of the enzyme. For the first time, we could show that, in astrocytes from rat brain, the transcription of COX subunit IV isoforms is regulated by the availability of oxygen, the substrate of the enzyme. In astrocytes, the lack of oxygen leads to a switch in the expression pattern from subunit IV-1 isoform under normoxic conditions to subunit IV-2 isoform under hypoxia. The functional consequences of the COX isoform expression on astrocytic energy metabolism under hypoxic conditions will be studied in more detail.

Selected Publications

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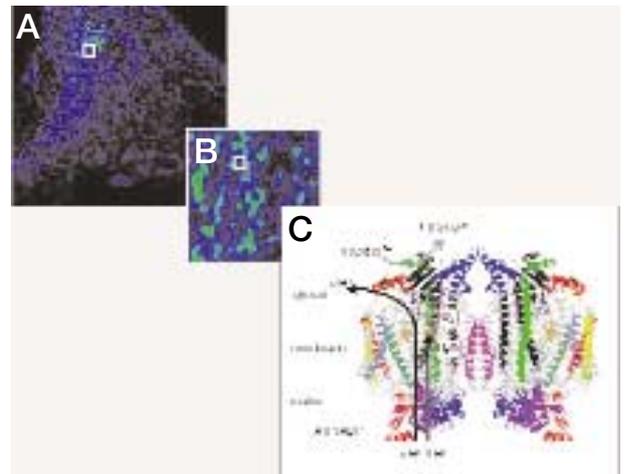
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A) B) Two photon microscope image of rhodamine 123 stained mitochondria from mouse astrocytes.
C) Structure of the dimeric cytochrome c oxidase complex from bovine heart [Tsukihara et al. (1996) *Science* 272, 1136]
Coordinates (PDB entry 1ocq) were processed with Raswin 2.6.



Developmental Neurobiology

Fritz G. Rathjen

Activity-independent and -dependent processes regulate the wiring of the nervous system

The establishment of precise and selective synaptic connections between neurons during embryonic and early postnatal development is essential for the proper functioning of the nervous system. A longstanding goal of many neuroscientists is to understand the formation of these neuronal connections throughout the nervous system which most likely involves a complex series of events. However, in principle, two overlapping mechanisms might be distinguished: processes that are independent of electric activity and processes that are dependent on electric activity of the neurons. Neuroscientists assume that activity-independent mechanisms establish the basic pattern of connectivity that becomes subsequently refined by activity-dependent processes.

Activity-independent molecular processes have been analyzed in detail in past years and several molecular guidance cues in the environment of axons, as well as guidance receptors on the surface of growth cones, have been characterized. For a general overview, the activity-independent axonal guidance factors have been categorized as being attractive or repulsive to growth cones. These factors belong also to different protein families including neural members of the Ig superfamily, semaphorins, netrins, ephrins, neuropilins, plexins, Eph-kinases and several extracellular matrix proteins. Our previous work focussed primarily on several Ig superfamily members such as F11, L1, neurofascin, NrCAM and neurotractin.

In contrast to the activity-independent mechanisms, the specific manner in which electric activity influences the refinement of synapses is still a matter of speculation and is controversial even in relatively well-established systems such as the visual system.

In the past granting period, the research activities of our group focussed on different aspects of axonal pathfinding regulated by activity-independent processes including cGMP

signalling, characterization of specific axonal Ig superfamily members, and induction of actin-rich microprocesses along axons. Since activity-dependent processes are less understood, we became also interested in identifying cell surface proteins on neurons which are modulated by neuronal activity.

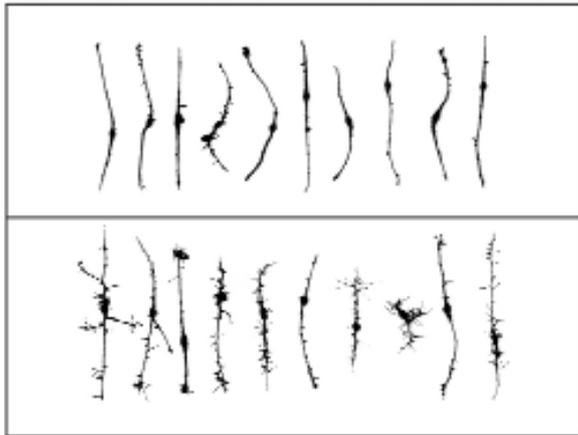
Tenascin-R induces microprocesses along neurite shafts

During development, axons and dendrites generate finger-like protrusions called filopodia or microprocesses along their shafts. Several observations have led to the proposal that these filopodia most likely initiate synapse formation by reaching out to neighbouring neurites and, therefore, might play an inductive role in the generation of synapses. In a search for extracellular signals that affect the formation of microprocesses in tectal neurons, we identified Tenascin-R (TN-R), a glycoprotein of the extracellular matrix. The formation of microprocesses by TN-R extending laterally along the neuritic shaft was time and dose-dependent and was accompanied by a rearrangement of the cytoskeleton. Contactin (F11), a cell adhesion molecule of the Ig superfamily that is associated with the plasma membrane via a lipid anchor, was found to mediate the effects of TN-R. These observations suggest that TN-R might induce a transition from long distance growth of tectal interneurons to differentiation, including the formation of microprocesses. Current studies focus on the intracellular signal transduction cascade activated by TN-R via contactin to induce filopodia along neurite shafts.

cGMP-mediated signalling in sensory axon pathfinding within the spinal cord

The signal transduction machinery within the growth cone can be modulated by the levels of the cyclic nucleotides cAMP and cGMP. Our studies on the pathfinding of sensory axons indicated that the cGMP-dependent protein kinase I (cGKI) is required for sensory axons to find their way within the dorsal root entry zone (DREZ) of the spinal cord. Once sensory axons arrive at the DREZ of the spinal cord, each axon bifurcates into a rostral and caudal branch extending over several segments. After a waiting period, collaterals grow out from these longitudinal stem axons and form lamina-specific projections within the grey matter. In the absence of cGKI, we observed, by DiI tracing and antibody staining, that many sensory axons fail to bifurcate correctly at the DREZ and instead grow directly to the central canal. These axon guidance defects in cGKI-deficient mice result in a substantial reduction of the amplitude of the nociceptive flexion reflex. Our studies, therefore, demonstrated that cGMP signalling via cGKI is important for directing axonal growth of sensory axons.

To further our understanding of these observations, we are currently investigating up- and downstream components of the cGKI-signalling pathway within sensory axons. We are using biochemical approaches, in vitro outgrowth studies, as well as genetic mouse models to unravel components of the cGMP signalling cascade within sensory axons. This includes



Induction of filopodia on neurites by the extracellular matrix glycoprotein Tenascin-R (TN-R). These highly dynamic microprocesses might be important for initial contacts to neighbouring neurites to establish synapses.

the characterization of guidance signals acting within the DREZ as well as their corresponding guidance receptor(s) on sensory axons whose activation elevates intracellular cGMP levels and, subsequently, triggers cGKI. The identification of the soluble or particulate guanylyl cyclases, which are involved in the generation of cGMP and the phosphodiesterase(s) which degrade cGMP, are also of primary interest. The analysis of downstream targets which are phosphorylated by cGKI should also help to define the function of cGKI in sensory growth cone steering further. Finally, we will apply our knowledge about cGMP signalling in sensory axons to other relevant neuronal cell populations in the central nervous system that express cGKI during development.

The proteolytical processing of CALEB is facilitated by electric activity and in the absence of CALEB synapse formation is impaired

As described above, the formation of precise synaptic connections in the brain is critically dependent on electric activity, which is important for the elimination of inappropriate connections and for the fine-tuning and stabilization of appropriate ones. The molecular constituents mediating these processes are largely unknown. In an attempt to characterize components by which electric activity influences the development of synapses, we have searched for cell surface proteins modulated by neural activity. To date, we have identified two proteins, termed CALEB and CAR, modulated on the surface of neurons by activity-dependent processes. Further studies have revealed that neuronal activity facilitates the proteolytical processing of the transmembrane protein CALEB resulting in a membrane associated form with an exposed EGF domain. In order to study the role of CALEB in synapse formation, a CALEB-deficient mouse was generated. The analysis of acute slices of the colliculus superior from these mice revealed synapses functionally distinct from wild-type synapses at early but not at mature stages. CALEB-deficient synapses revealed a reduction of the frequency of spontaneous EPSCs and IPSCs which are due to deficits within the pre-synapse. These findings indicate that CALEB signalling is required for the molecular development of synapses in early stages.

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Growth Factors and Regeneration

Gary Lewin

Sensory neurons of the dorsal root ganglia allow us to 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. In addition, we also study the genetic programs controlled by growth factors that specify sensory neuron function or their connections with spinal cord circuits.

Molecular Basis of Mechanotransduction

Role of mec genes

Mechanotransduction is the process whereby receptor proteins present in the endings of sensory neurons are able to detect mechanical stimulation of the tissue they innervate. We have used information from genetic experiments with the nematode worm *C.elegans* to identify possible vertebrate candidate proteins that might detect mechanical stimuli. Genetic screens for touch insensitive worms have turned up around 15 genes whose function is necessary to confer touch sensitivity. These genes were named Mec for mechanically insensitive and we have focused on identifying a role mammalian orthologs of these genes in mammalian touch sensation. Some of these genes encoded membrane ion channels that were proposed to open upon movement or displacement of the plasma membrane. We have recently shown that a mouse protein (BNC1/ASIC2) with significant homology to the worm ion channels is required for mice to properly discriminate touch stimuli (Price et al. 2000). Other work in the lab has concentrated on establishing whether the BNC1/ASIC2 ion channel works in concert with other ion channel subunits (eg. the DRASIC protein) to detect mechanical forces (Price et al. 2001). 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 also the mammalian

orthologs of mec-2 are essential for normal mechanotransduction.

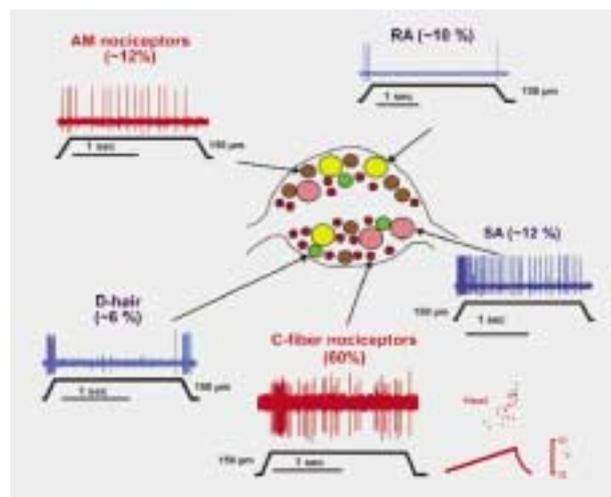
Mining the genome for sensory neuron markers

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 (Stucky et al. 2002a). 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 (Shin et al. 2003). We are extending this approach at the moment to find functionally important marker genes of other mechanoreceptor types including nociceptors.

Transduction in a dish

In addition to examining the function of candidate genes in sensory transduction the laboratory is also using electrophysiological techniques to characterize sensory transduction in single cells. For example, we use cultured sensory neurons to characterize with whole cell patch clamp techniques the ionic currents underlying thermal stimuli (Stucky et al. 2002b). Recently, we have established methodologies to measure

Different mechanoreceptor types in the dorsal root ganglion. In the middle is a schematic drawing of the dorsal root ganglia that contains the cells bodies of the sensory neurons. Neurons are colour coded according to their sensory modality. For each neuronal type a typical recording of the response to a ramp and hold indentation of the skin is shown. The responses of neuronal types that detect small non-painful stimuli (touch receptors) are coloured blue and those detecting intense noxious stimuli are shown in red (pain receptors).



mechanically-gated ionic conductances in single sensory neurons and we are investigating the effect of extracellular matrix factors on these channels.

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.

Regulation of sensory synaptic connections in the spinal cord

The synaptic connections made by sensory neurons in the spinal cord underlie reflexes evoked by innocuous or noxious stimuli. We have recently developed an *in vitro* electrophysiological preparation to study such reflexes in the mouse (Pesquero et al., 2000). This technique, together with the use of knockout mice, recently allowed us to identify the neurotrophin, Brain derived neurotrophic factor (BDNF) as a functionally important pain neuromodulator released by sensory neurons onto the spinal cord neurons (Heppenstall and Lewin 2001). In collaboration with other MDC groups, we have developed this technique to provide high quality phenotype information on many different mouse mutants to identify genes regulating the construction or function of spinal reflexes (Müller et al. 2002; Schmidt et al. 2002). This data will provide insights into the genes needed to construct the somatosensory system and possibly reveal new drug targets for the treatment of acute and chronic pain.

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Proteomics and Molecular Mechanisms of Neurodegenerative Disorders

Erich Wanker

The sequencing of the human genome has provided the basis for the systematic analysis of protein function. The main objective of our work is to generate knowledge about human proteins and to link them to disease processes using high-throughput functional proteomics technologies. Through the large-scale identification of physical protein-protein interactions (PPIs) by automated yeast two-hybrid screening, we are creating comprehensive protein interaction maps in order to characterise proteins and to understand the regulatory processes that control the biology of living organisms. These studies are also intended to identify new targets for therapeutic intervention. Closely linked to these goals is our work on the function and dysfunction of proteins involved in late onset neurodegenerative diseases like Huntington's (HD), Parkinson's (PD) and Alzheimer's (AD) disease. These disorders are characterised by the accumulation of intra- and extracellular protein aggregates, considered critical for disease pathogenesis. We have developed cell-based and *in vitro* drug screening assays for the identification of small molecules that delay aggregate formation. Lead compounds have been identified and are currently tested for their activity in different transgenic *in vivo* model systems.

Generation of a protein-protein interaction network for Huntington's disease by automated two-hybrid screening

In the past two years, we have developed a strategy combining library and matrix yeast two-hybrid screens to create a highly connected protein-protein interaction network for Huntington's disease (HD). We used 40 proteins with 10 different huntingtin (htt) fragments for library screens and tested all identified proteins for interactions with all bait proteins in a systematic array-mating screen. In total, a network consisting of 86 proteins involved in 188 protein-protein interactions was generated, 16 of which were confirmed by *in vitro* binding assays, co-immunoprecipitations, or co-localisation studies. Sixteen uncharacterised proteins could be functionally annotated and a G protein-coupled receptor kinase

interacting protein, GIT1, was discovered. In HD patient brains, GIT1 is present in neuronal inclusions, suggesting that it is involved in disease pathogenesis.

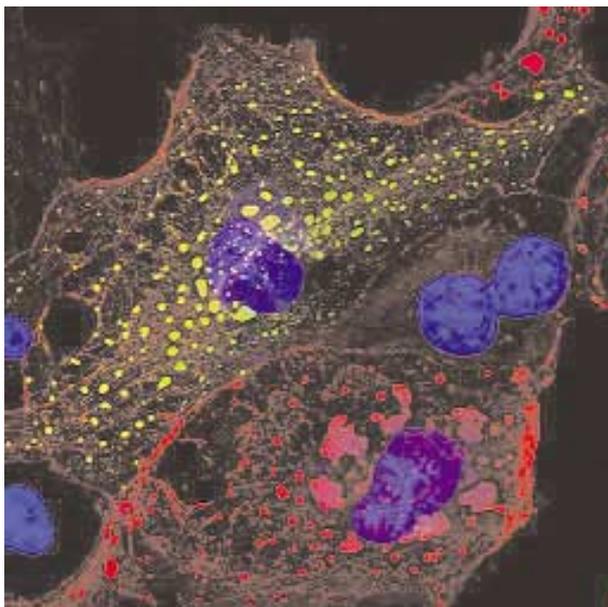
Systematic approach: Large scale mating two-hybrid screens

Gene and protein function have become the major areas of inquiry on the way to a full description of all biological processes and their malfunction. Proteins rarely act alone in the cell. Their interaction patterns provide valuable information about their function. We are currently generating a comprehensive human protein-protein interaction network using an automated yeast two-hybrid (Y2H) system. This large-scale, systematic study is based on interaction mating in a 384-well matrix format. Two different haploid yeast strains of opposite mating type (MAT α and MATa) are mated after transformation with plasmids encoding activation domain and DNA-binding domain fusion proteins, respectively. Interactions are detected via transcriptional reporter activation. A non-redundant set of more than 3,600 cDNAs has been subcloned into DNA-binding and activation domain vectors. Additionally, 2,000 human full length ORFs were cloned into two-hybrid vectors. To handle the large number of yeast clones, an automated version of the Y2H system was set up, using pipetting, spotting, and gridding robots. A screening of about 25 million potential interactions was performed and allowed the initial identification of about 2,000 PPIs. These interactions are currently validated and verified using bioinformatics, cell biology, and biochemical methods with special attention to disease proteins.

High-throughput protein expression and production of protein arrays

Recombinant human proteins are valuable resources for many applications in functional genomics and proteomics. We have developed an approach that allows the parallel expression of ~13,500 His-tagged fusion proteins from a human brain cDNA library in 384-well microtitre plates. Crude protein extracts are high-density gridded onto 22 x 22 cm membrane filters with a spotting robot. We then probed the arrays with an anti-RGS-His antibody and identified about 2,300 recombinant human proteins available for further interaction studies.

The high-density spotted membranes were probed with bacterial protein extracts containing overexpressed recombinant human GST fusion proteins as baits. Reproducible interaction maps were obtained for 15 bait proteins involved in neurodegenerative diseases, endocytosis, and gene expression. In total, the interaction studies with high-density spotted protein arrays allowed the identification of about 150 PPIs, which are currently validated by means of pull-down assays, mass spectrometry, co-localisation experiments and functional assays.



Immunofluorescence microscopy image of COS1 cells expressing GIT1 and huntingtin. GIT1 was identified by automated yeast two-hybrid screening using huntingtin as bait. It binds to huntingtin and stimulates the accumulation of membrane vesicles in early endosomes. Moreover, GIT1 promotes the accumulation of huntingtin aggregates in mammalian cells.

Co-aggregation of mutant and wild-type huntingtin

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by an expanded polyQ repeat in the N-terminal part of the htt protein. The elongated protein self-assembles into amyloid-like aggregates, while wild-type htt does not aggregate. Using cell-free and cell-based assays, we found that mutant htt promotes the aggregation of wild-type htt by a nucleation-dependent process, causing the formation of SDS-resistant co-aggregates with a fibrillar morphology. Conversely, mutant htt does not promote the fibrillogenesis of the polyQ-containing protein NOCT3 or the polyQ-binding protein PQBP1, although they are recruited into inclusions containing mutant htt aggregates in mammalian cells. Htt fibril formation is a highly selective process, depending on polyQ tract length and on other determinants in the htt amino acid sequence. Our data suggest that mutant and wild-type htt also co-aggregate in neurons of HD patients and that the resulting loss of wild-type htt function may contribute to pathogenesis.

P97 selectively mitigates ataxin-3 aggregation and toxicity in *Drosophila*

Spinocerebellar ataxia type 3 (SCA3) is an autosomal, dominantly inherited neurodegenerative disorder caused by an expanded polyQ sequence in the ataxin-3 protein. Function of the protein and the molecular mechanism of SCA3 are unknown. We have isolated p97, a AAA ATPase, copurifying with ataxin-3. Protein p97 selectively binds to ataxin-3 but not to other polyQ-containing proteins such as htt, indicating that it is not a polyQ-binding protein. In a series of experiments, we found that a 70 amino acid ataxin-3 fragment was

necessary and sufficient for the binding to p97. It is localised downstream of the polyQ tract and contains two putative ubiquitin interacting motifs (UIMs). The p97 N-terminal domain (aa 1-200) was crucial for the ataxin-3 interaction.

Testing whether p97 modulates ataxin-3 aggregation, we found that increasing concentrations of p97 up to equimolarity stimulated it, whereas a 3 to 4-fold molar excess of p97 strongly suppressed it. This was confirmed in a cellular model. In a transgenic *Drosophila* model of SCA3, both overexpression of human p97 and heterozygous loss of the protein suppressed polyglutamine induced neurodegeneration. Moreover, neuronal intranuclear inclusions containing aggregated ataxin-3 showed a significant size reduction. Alteration of p97 levels in neurons could open up new avenues for therapeutic intervention in SCA3.

Characterisation of amyloid aggregation inhibitors using *in vitro* and *in vivo* model systems

Neuronal protein aggregates are a hallmark of neurodegenerative disorders, like Alzheimer's (AD), Parkinson's (PD), and Huntington's disease (HD). Animal studies suggest that removal of aggregates ameliorates the disease course. Especially for HD, it is now widely assumed that aggregation inhibition in patients will slow down disease progression.

To search for polyQ aggregation inhibiting compounds, we performed a screen using an automated filter retardation assay. We detected around 300 compounds, a major group being benzothiazoles, significantly reducing htt aggregate formation in a dose-dependent manner. Some were then found to inhibit polyQ aggregation in mammalian cells. Currently, cytotoxicity assays are conducted. The most promising compounds will then be studied in transgenic mouse models of HD. The HD R6/2 transgenic mouse model was established for systematic drug screens. The mice are transgenic for exon 1 of the human HD gene with a greatly expanded CAG repeat and recapitulate many of the features of the human disease. At the moment, the benzothiazole derivatives PGL-135 and PGL-137 are studied. The effects are assessed in behavioural assays like motor ability and grip strength tests. Brains are examined immunohistologically and htt aggregates are quantified using a filter retardation assay. Compounds able to reduce aggregate formation and neurological symptoms in these mice will be selected as candidates for clinical trials.

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Neurodegeneration

Christiane Alexander

Autosomal dominant optic atrophy (adOA) is the most prevalent hereditary optic neuropathy in humans resulting in a progressive loss of visual acuity, centrocoecal scotoma, and bilateral temporal atrophy of the optic nerve with an onset within the first two decades of life. adOA occurs with an estimated disease prevalence of between 1:12,000 (Denmark) and 1:50,000. The disease is highly variable in expression and shows incomplete penetrance in some families. 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.

OPA1 – the gene causing autosomal dominant optic atrophy

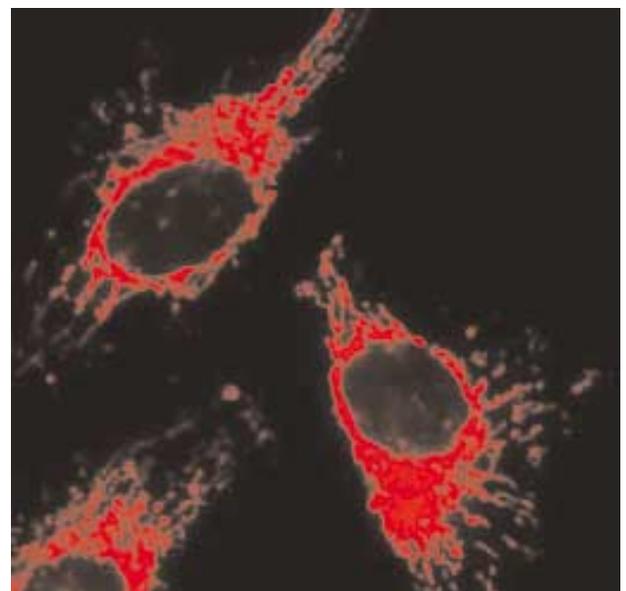
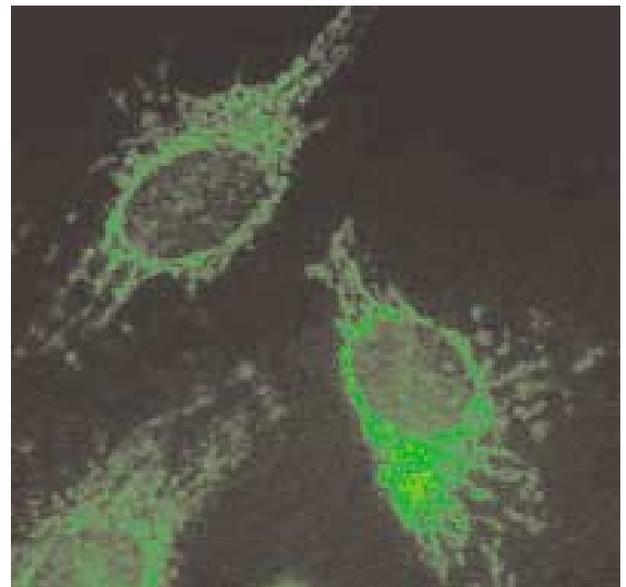
The predominant locus for this disorder (*OPA1*, OMIM #165500) was mapped by linkage analysis in large Danish pedigrees to a 1.4 cM interval on chromosome 3q28-q29 flanked by markers *D3S3669* and *D3S3562*. By positional cloning, we were able to identify the underlying disease gene, *OPA1*, which spans about 100 kb of genomic sequence and is divided into 31 exons. The human *OPA1* (*hOPA1*) cDNA was first described with a length of 5864 bp containing an open reading frame (ORF) of 2883 bp. 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 hot-spot involving exons 4, 4b, 5 and 5b was discovered recently, without evidence for mutations being identified in these exons in any *OPA1* patients screened, so far.

The *OPA1* GTPase is a mitochondrial protein

The *OPA1* protein consists of a classical N-terminal mitochondrial import signal, a coiled-coil domain (CC1), a GTPase, a middle domain of unknown function, and a C-terminal coiled-coil domain (CC2). *OPA1* shows homology to dynamin-related large GTPases from salmon, *C.elegans*, *Drosophila*, and the rat. Dynamin-related GTPases are involved in fusion and fission processes of membranes everywhere in eucaryotic cells, f.e. vesicle formation at the plasma membrane, the endosome, or at the golgi apparatus. A novel aspect of recently identified members of the Dynamin protein family, like *OPA1*, *DNM1* and *Mitofusins*, is their involvement in the fusion and fragmentation of mitochondria. Defects in these proteins lead to an abnormal distribution of mitochondria in the cell, as well as to deficits in mitochondrial function, f.e. respiration, or apoptosis. First ideas on the function of *Dnm1* and *Mitofusins* have been developed, whereas for *OPA1* elucidation of its role in the cell and in the nervous system remains to be established. We are gaining first insights into *OPA1* function by the identification of interaction partners via yeast-two-hybrid screens, the structural characterization of *OPA1* protein domains, and the generation of animal models.

Visualization of mitochondria.

Immunostaining of HeLa cells with anti-cytochrome C antibody (green) and simultaneous live staining of the same cells with Mitotracker (red).



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.

Analysis of a Drosophila OPA1-model

In *Drosophila*, the genetic regulation of the visual system has been successfully studied in the past, proving that the fly is an excellent model for ocular diseases affecting photoreceptor cells in humans. 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 15 exons. Moreover, we discovered splice variants of the fly *OPA1* mRNA similar to the transcripts generated by the splicing hot spots in humans and mice.

As part of a large-scale approach to study gene function in *Drosophila*, P-element insertion lines have been created. In one of these lines, a P-element happened to be inserted into exon 2 of the *Drosophila* *OPA1* gene leading to the functional disruption of this chromosomal gene allele. First studies of this fly line revealed that, while heterozygous flies are seemingly perfectly viable, homozygous animals die at a late larval stage. Transgenic flies that are being created in our lab will help to dissect the importance of *OPA1* function for the nervous system in comparison to other tissues.

Selected Publications

Delettre C, Lenaers G, Pelloquin L, Belenguer P, Hamel C P (2002). *OPA1* (Kjer Type) Dominant Optic Atrophy: A Novel Mitochondrial Disease. *Mol. Genet. Metabol.* 75, 97-107.

Thiselton DL, Alexander C, Taanman JW, Brooks S, Rosenberg T, Eiberg H, Andreasson S, Van Regemorter N, Munier FL, Moore AT, Bhattacharya SS, Votruba M (2002). A comprehensive survey of mutations in the *OPA1* gene in patients with autosomal dominant optic atrophy. *Invest Ophthalmol Vis Sci.* 43(6):1715-24.

Aung T, Ocaka L, Ebenezer ND, Morris AG, Krawczak M, Thiselton DL, Alexander C, Votruba M, Brice G, Child AH, Francis PJ, Hitchings RA, Lehmann OJ, Bhattacharya SS (2002). A major marker for normal tension glaucoma: association with polymorphisms in the *OPA1* gene. *Hum Genet.* 110(1):52-6.

Pesch, UE, Leo-Kottler, B, Mayer, S, Jurklies, B, Kellner, U, Apfelstedt-Sylla, E, Zrenner, E, Alexander, C, Wissinger, B. (2001). *OPA1* mutations in patients with autosomal dominant optic atrophy and evidence for semi-dominant inheritance. *Hum. Mol. Genet.* 10(13):1359-68.

Alexander, C, Votruba, M, Pesch UEA, Thiselton, DL, Mayer, S, Moore, A, Rodriguez, M, Kellner, U, Leo-Kottler, B, Auburger, G, Bhattacharya, SS, Wissinger, B. (2000). *OPA1*, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nature Genet.* 26, 211-215.

Structure of the Group

Group Leader

Dr. Christiane Alexander

Graduate Students

Maja Fiket

Dr. Vasudheva Reddy Akepati

Marco DelBarba

Anita Bulczak

Technical Assistants

Iska Liebner

Neuronal Stem Cells

Gerd Kempermann

The adult hippocampus, a brain region centrally involved in learning and memory processes, produces new neurons throughout life. This “adult neurogenesis” is regulated in an activity-dependent manner and originates from neuronal stem cells. We are interested in the biology of neuronal stem and progenitor cells in the adult brain and their contribution to brain function in health and disease. We would like to show how new neurons contribute to normal hippocampal function and if and how a failure of adult neurogenesis could be involved in the pathogenesis of complex disorders such as memory loss, temporal lobe epilepsy, major depression, or Alzheimer’s disease. Our hypothesis is that adult neurogenesis and its activity-dependent regulation allow the brain to optimize the strengths of the mossy fiber tract, an important pathway in the hippocampus, according to functional needs. Beyond these hippocampus-specific aims, we can learn from adult hippocampal neurogenesis about how the development of new neurons is possible under the conditions of the adult brain. Except for the hippocampus and the olfactory system, the adult brain does not promote the generation of new neurons. This is one reason why many neurological disorders are chronic and incurable. Our central research questions are what makes the hippocampus a neurogenic region and how can we define this neurogenic permissiveness on a cellular and molecular level.

Characterizing stem cells and neuronal development in the adult brain

Knowledge about the identity, the potential, and the function of neuronal stem cells is still scarce. We have found that the putative stem cells of the hippocampus show glial features, thereby relating them to radial glia and astrocytes. However, the population of proliferating cells that is affected by stimuli regulating adult neurogenesis is a distinct group of progenitor cells lacking glial features and showing among itself a high degree of heterogeneity. Whereas the stem cells carry the general regenerative potential, it might be that the regulation of other progenitor cell populations actually allows neuro-

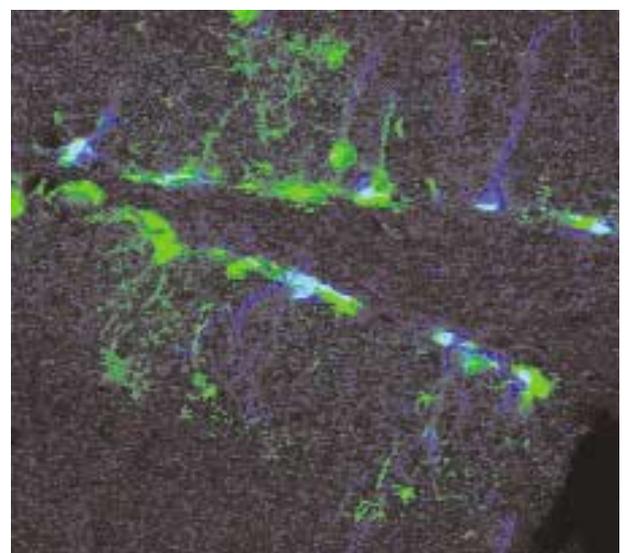
genesis and activity-dependent cellular plasticity to occur. Our *in vivo* studies have shown that in the dentate gyrus alone, four distinct cell types with progenitor properties can be identified. We would like to know during which of these stages the actual fate choice decision for neuronal development is made and how it is regulated. We have also characterized other stages of neuronal development in the adult hippocampus. Once the immature neurons have left the cell cycle, they go through a transient stage, during which they express the calcium binding protein calretinin. Most likely, this expression marks the important developmental phase during which the new neurons extend their axon and establish synaptic contacts. Over a period of several weeks, the new cells mature into their ability to respond to a synaptic stimulation and thus become functional as neurons.

Searching for the regulatory master genes

Comparative studies in different strains of mice have revealed that adult hippocampal neurogenesis is regulated on several regulatory levels, which are differentially influenced by inherited traits. In a linkage study in sixty strains of mice, we searched for key gene loci associated with the baseline level of adult hippocampal neurogenesis in mice. Interestingly, we found that the baseline level of neurogenesis was correlated with parameters describing the acquisition of a hippocampus-dependent learning task. We identified three candidate loci on two chromosomes and are currently trying to identify candidate genes in these regions.

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 located at the Dept. of Neurology, Charité

The putative stem cells of the adult hippocampus have a surprising, tree-like morphology (green). This appearance resembles radial glia, which plays a fundamental role during embryonic brain development. The cells that are labeled blue in the microscopic image fall into two categories. Some of them have only short processes: these are progenitor cells that are still proliferative. The other type are immature neurons that have already extended their dendritic process towards the molecular layer of the dentate gyrus - the same area where we find the delicate arborization of the radial glia-like stem cells.



University of Medicine Berlin. The goal of this interaction is to allow clinicians to pursue stem cell research in close interaction with the clinic, but within the scientific environment provided by the MDC.

Selected Publications

Kronenberg, G., Reuter, K., Steiner, B., Brandt, M.D., Jessberger, S., Yamaguchi, M., Kempermann G. (2003). Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *Journal of Comparative Neurology* 467, 455-463.

Brandt, M., Jessberger, S., Steiner, B., Kronenberg, G., Reuter, K., Bick-Sander, A., von der Behrens, W., Kempermann, G. (2003). Transient calretinin-expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. *Molecular and Cellular Neuroscience* 24, 603-613.

Ehninger, D., Kempermann, G. (2003). Regional differences of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. *Cerebral Cortex* 13, 845-851.

Filippov, V., Kronenberg, G., Pivneva, T., Reuter, K., Steiner, B., Wang, L.P., Yamaguchi, M., Kettenmann, H., Kempermann, G. (2003). Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Molecular and Cellular Neuroscience* 23, 373-382.

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., Gage, F.H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 130, 391-399.

Structure of the Group

Group Leader

Dr. Gerd Kempermann

Scientists

Dr. Gudrun Lutsch

Dr. Golo Kronenberg

Dr. Anika Bick-Sander

Dr. Barbara Steiner

Dr. Sebastian Jeßberger

Dr. Susanne Wolf

Dr. Gerold Brüning (visiting scientist)

Graduate and Undergraduate Students

Dan Ehninger

Moritz Brandt

Ana Garcia

Harish Babu

Kathrin Lehmann

Benedikt Römer

Frank Rolfs

Technical Assistants

Irene Thun

Daniela Gast

Ruth Segner

Ulrike Ziegler

Erika Kotitschke

Bioethics and Science Communication

Christof Tannert

Project Scope

The interdisciplinary working group, funded by the BMBF and the MDC, in co-operation with the *Forschungszentrum Jülich* (FZJ), analyses and develops the societal discourse on biomedicine. Two main aspects form the center piece of the work: (1) The ethical questions that arise in the context of biomedical research and (2) communication of risks and benefits associated with biomedicine. We aim to improve communication between science, politics, media, and the public as well as help to openly debate critical questions about the opportunities and risks associated with biomedicine. We examine how the preconditions for “force (of the better argument) without coercion” can be created. This includes the comprehensibility and completeness of scientific-technical information, the clarification of uncertainties within risk, and opportunity assessments as well as the building of trust on the basis of a fair and open dialogue.

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

Delphi Study

A study on the Future of Stem Cell Research in Germany, referred to as the ‘Delphi Study’, was conducted. Following standard procedure, a postal questionnaire with 57 hypotheses on the future of basic and applied research on embryonic and adult stem cells as well as relevant societal aspects was administered twice to leading German stem cell experts. These Experts had to assess the timeframe of realisation of each hypothesis, the respective risks and opportunities for patients, research and industry, as well as the most relevant factors influencing the advancement of research in Germany. The study aims to show trends and time horizons as well as basic parameters and important local/national conditions for stem cell research. The results will be published in Spring 2004.

Online-Conference

An Online-Conference of relevant stakeholders was held from September to November 2003 and involved discussion of the social, ethical, and economic aspects of the research. Its aim was to (1) pilot the use of internet based dialogue forums and (2) to engage stakeholders in a meaningful exchange about the implications of the future of stem cell research. The interface between basic research and its clinical application was highlighted as an important area for further research. The role of the clinician in steering the transfer of research findings into clinical best practice was seen as a key aspect.

Citizens' Conference

A Citizens' Conference was held in March 2004 following the Danish model of consensus conferences. A group of 15 lay people was randomly selected to collaborate and produce a “Citizens' Vote” on the social and ethical aspects of stem cell research. On two weekends in 2003/2004, the group intensively worked on the relevant subject areas to come up with a list of questions. These were presented to a group of thirteen experts from science, ethics, policy, and business in a public expert hearing at the Berlin-Brandenburg Academy of the Sciences. On the basis of the hearing and their previous work, the group wrote a statement and presented it to the President of the Deutsche Bundestag, Wolfgang Thierse.

Internet Presence

The Interactive website (www.bioethik-diskurs.de) was established in October 2002 and is continually updated. A barrier-free version is available for disabled persons. Monthly visitor numbers average around 2,200 (November 2003).

Selected Publications

J. Niewöhner, P. Wiedemann, C. Karger, S. Schicktanz, C. Tannert: “Participatory prognosis in Germany – developing citizen scenarios for the relationship between biomedicine and the economy in 2014” (in press) *J. Technological Forecasting and Social Change*

A. Görzdorf und C. Tannert: “Die Szenario-Methode als Verfahren der Bürgerbeteiligung? Stärken, Schwächen, Möglichkeiten und Problemquellen aus Sicht von Teilnehmern des Workshops ‘Die Grenzen von Genen, Geld und Gelehrten’” (in press) *Zschr. Biopolitik*

S. Schicktanz, C. Tannert und P. Wiedemann (Eds.): “Kulturelle Aspekte der Biomedizin: Bioethik, Religionen und Alltagsperspektiven” Campus-Verlag FfM (2003) Series Edition “Kultur der Medizin”, 304 pages, ISBN 3-593-37388-2

C. Tannert: “Biologische und theologische Anmerkungen zum deutschen Ethik-Diskurs zur Biomedizin” (2003) *Zschr. Biopolitik* 2(3), 185

S. Domasch: "Zukunfts-Szenarien. Szenario-Workshop."
(2003) GenomXpress 1(03),18

S. Domasch und C. Tannert: "Transparenz und Erfahrbarkeit
von Wissen – Bioethik und Wissenschaftskommunikation."
(2002) Zschr. Humanontogenetik 5(2), 82

Structure of the Group

Group Leader
Dr. Christof Tannert

Collaborating Group Leader (FZJ)
Dr. Peter Wiedemann

Scientists
Dr. Susanne Reif
Dr. Jörg Niewöhner

Associate Scientists
Dr. Silke Schicktanz
Judith Simon

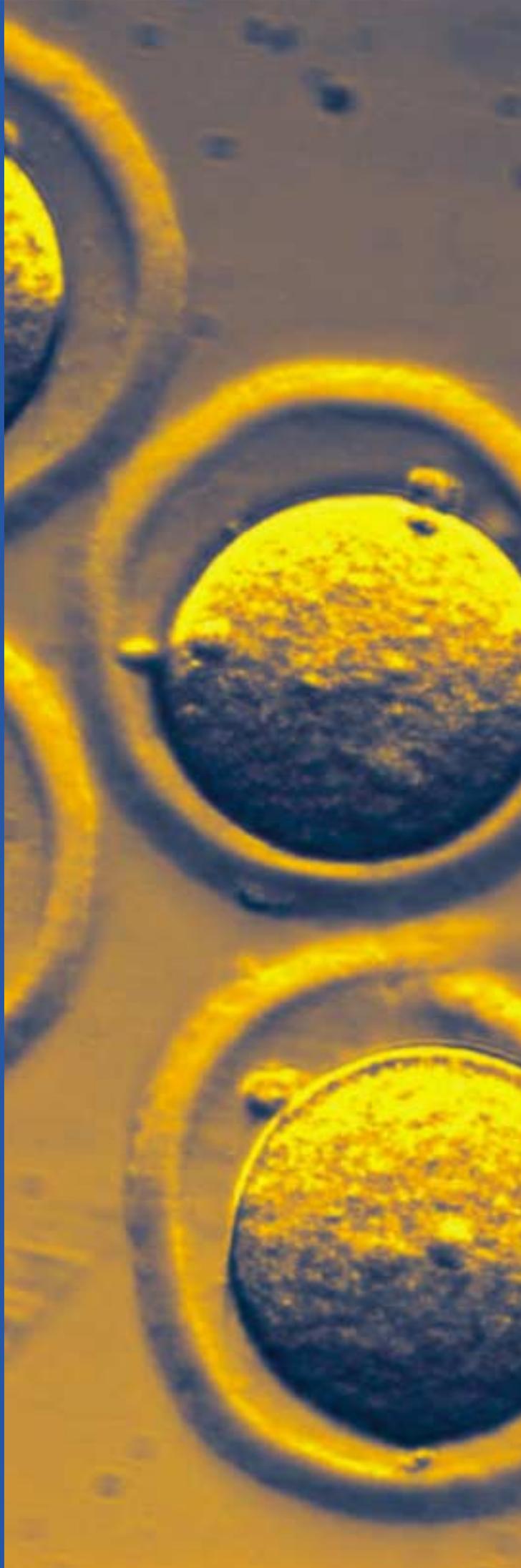
Guest Scientists
Prof. Dr. Philippe Meyer
Dr. Norbert Paul

Management Assistant
Ali ben Salem

Secretarial Office
Ulrike Zehlike

Student Support
Lars Kaufmann
Mark Schweda

Academics



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 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.

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. As a result, the first joint advertisement for junior professorships in the field of Medical Genomics was published in the Spring of 2003. Thus, for the first time, two junior professors, Prof. Dr. Michael Gotthardt and Prof. Dr. Norbert Hübner, were appointed during the period of the report.

In addition, the following academic appointments were made:

Prof. Dr. Carmen Birchmeier-Kohler, MDC Head of the Research Group Developmental Biology/Signal Transduction in Nerve and Muscle Cells, accepted the offer from the MDC and the Free University Berlin of a C4-S-Professorship in Molecular Therapy, Signal Transduction, and Developmental Biology in 2002.

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/Diplomandinnen bzw. Doktoranden/Doktorandinnen zu den Berliner Universitäten sichern und ihren Mitarbeiterinnen und Mitarbeitern die Möglichkeit eröffnen, an den entsprechenden Fakultäten zu promovieren und sich zu habilitieren.

Seit 2002 kann das MDC auf Grundlage des 5. Änderungsgesetzes zum Hochschulrahmengesetz gemeinsam mit Berliner Universitäten Junior-Professoren berufen. Auf der Grundlage der Vereinbarung über die Zusammenarbeit zwischen dem MDC und der Humboldt-Universität zu Berlin aus dem Jahre 1994 wurde 2002 eine Ergänzungsvereinbarung abgeschlossen, die die Ernennung von Junior-Professoren analog der Leitsätze für gemeinsame Berufung auf konventionelle Professuren ermöglicht. Das MDC hat im Dezember 2002 in gleicher Weise mit der Freien Universität Berlin eine Ergänzungsvereinbarung beschlossen. Eine erste gemeinsame Ausschreibung auf Junior-Professuren im Bereich Medical Genomics wurde im Frühjahr 2003 veröffentlicht. Mit Prof. Dr. Michael Gotthardt und Prof. Dr. Norbert Hübner konnten erstmalig zwei Junior-Professoren berufen werden.

Folgende Wissenschaftler sind berufen worden:

Prof. Dr. Carmen Birchmeier-Kohler, MDC-Forschungsgruppenleiterin in Entwicklungsbiologie/Signaltransduktion in Nerven und Muskelzellen, hat 2002 den Ruf des MDC und der Freien Universität Berlin auf die C4-S-Professur Molekulare Therapie, Signaltransduktion, Entwicklungsbiologie angenommen.



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Prof. Dr. Thomas Dandekar, MDC Research Group Bioinformatics (Prof. Jens Reich), accepted a chair of Bioinformatics in December 2002 at the Julius Maximilians University Würzburg.

Dr. Yasuyuki Fujita, MDC Research Group Epithelial Differentiation, Invasion, and Metastasis (Prof. Walter Birchmeier), accepted an offer as a junior group leader at the Medical Research Council (MRC) Laboratory for Molecular Cell Biology (University College London) in October 2002.

Dr. rer. nat. Jörg Hülsken, MDC Research Group Epithelial Differentiation, Invasion, and Metastasis (Prof. Walter Birchmeier), accepted an offer from the Swiss Institute for Experimental Cancer Research (Schweizerisches Institut für Experimentelle Krebsforschung (ISREC)) in Lausanne, Switzerland in January 2003.

Prof. Dr. Jens Jordan was appointed as a C3 Professor as the Head of the Clinical Research Center at the Charité University Medical School and Hospitals Berlin in September 2003.

Prof. Dr. Ralph Kettritz, Franz Volhard Clinic for Cardiovascular Diseases, Charité/Helios Teaching Hospitals Berlin, Nephrology and Hypertensiology, accepted a C3-Professorship of Nephrology at the Charité University Medical School Berlin in December 2003.

Prof. Dr. Thomas Dandekar, MDC-Forschungsgruppe Bioinformatik (Prof. Jens Reich), hat im Dezember 2002 einen Ruf auf den Lehrstuhl für Bioinformatik von der Julius-Maximilians-Universität Würzburg angenommen.

Dr. Yasuyuki Fujita, MDC-Forschungsgruppe Epitheliale Differenzierung, Invasivität und Metastasierung (Prof. Walter Birchmeier), hat im Oktober 2002 einen Ruf als Nachwuchsgruppenleiter vom Medical Research Council (MRC) Laboratory for Molecular Cell Biology (University College London) angenommen.

Dr. rer. nat. Jörg Hülsken, MDC-Forschungsgruppe Epitheliale Differenzierung, Invasivität und Metastasierung (Prof. Walter Birchmeier), hat im Januar 2003 einen Ruf als Nachwuchsgruppenleiter vom Schweizerischen Institut für Experimentelle Krebsforschung (ISREC) in Lausanne, Schweiz angenommen.

Prof. Dr. Jens Jordan hat im September 2003 einen Ruf auf die C3-Professur als Leiter des Clinical Research Center an die Charité-Universitätsmedizin Berlin angenommen.

Prof. Dr. Ralph Kettritz, Franz-Volhard-Klinik für Herz-Kreislaufkrankungen, Charité/Helios Klinikum Berlin, Nephrologie und Hypertensiologie, hat im Dezember 2003 einen Ruf auf die C3-Professur für Nephrologie der Charité-Universitätsmedizin Berlin angenommen.

Prof. Dr. Young-Ae Lee, Zentrum für Genkartierung des MDC und Kinderklinik der Charité der Humboldt-Universität zu Berlin, hat im November 2002 den Ruf auf eine Juniorprofessur des Faches Kinderheilkunde an der Charité angenommen. Prof. Dr. Lee's Forschungsgruppe ist am MDC angesiedelt.

Prof. Dr. Gary Lewin, Leiter der MDC-Forschungsgruppe Wachstumsfaktoren und Regeneration, hat im Sommer 2003 den Ruf des MDC und der Freien Universität Berlin auf eine C4-S-Professur angenommen.

Prof. Dr. Stefan Schuster, MDC-Forschungsgruppe Bioinformatik (Prof. Jens Reich), hat im April 2003 einen Ruf auf eine C3-Professur für Bioinformatik an die Universität Jena angenommen.

Prof. Dr. med. Arya Mitra Sharma, Franz-Volhard-Klinik für Herz-Kreislaufkrankungen, Charité/Helios Klinikum Berlin, MDC-Forschungsgruppenleiter Adipositas und Hypertonie, hat Ende 2002 einen Ruf der McMaster University, Hamilton/Ontario, Kanada angenommen.

Prof. Dr. Wolfgang Uckert ist 2002 auf die C3-S-Professur Molekulare Zellbiologie und Gentherapie der Mathematisch-Naturwissenschaftlichen Fakultät der Humboldt-Universität zu Berlin berufen worden. Er ist Nachfolger von Prof. Michael Strauss, der 1999 verstorben ist. Prof. Uckerts wissenschaftliche Arbeitsgruppe ist am MDC angesiedelt.

Prof. Dr. Young-Ae Lee, MDC Gene Mapping Center and Children's Hospital of the Charité of the Humboldt-University of Berlin, accepted a junior professorship in pediatrics at the Charité in November 2002. Prof. Dr. Lee's laboratory is located at the MDC.

Prof. Dr. Gary Lewin, Head of the MDC Research Group Growth Factor and Regeneration accepted the offer from the MDC and the Free University of Berlin of a C4-S-Professorship of Medical Genome Research in the Summer of 2003.

Prof. Dr. Stefan Schuster, MDC Research Group Bioinformatics (Prof. Jens Reich), accepted an offer of a C3-Professorship of Bioinformatics at the University of Jena in April 2003.

Prof. Dr. med. Arya Mitra Sharma, Franz Volhard Clinic for Cardiovascular Diseases, Charité/Helios Teaching Hospitals Berlin (Head of MDC Research Group Obesity and Hypertension), accepted an offer from the McMaster University, Hamilton/Ontario, Canada at the end of 2002.

Prof. Dr. Wolfgang Uckert was appointed to the C3-S-Professorship Molecular Cell Biology and Gene Therapy in 2002. He is the successor to Prof. Michael Strauss, who died in 1999. Prof. Uckert's research group is located at the MDC.

Prof. Dr. Rüdiger von Harsdorf, Franz Volhard Clinic for Cardiovascular Diseases, Charité/Helios Teaching Hospitals Berlin, and MDC Research Group Molecular Basis of Congestive Heart Failure, accepted a C3-Professorship for the specialist field Internal Medicine with a concentration in Cardiology at the Charité at the end of 2002.

Prof. Dr. Martin Zenke, MDC Research Group Molecular and Cell Biology of Hematopoietic Cells, was appointed as C4 Professor of Cell Biology and Director of the Helmholtz Institute for Biomedical Technology at the University Teaching Hospitals of the Rheinisch-Westfälischen Technischen Hochschule (RWTH) Aachen in November 2003.



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Prof. Dr. Rüdiger von Harsdorf, Franz-Volhard-Klinik für Herz-Kreislaufkrankungen, Charité/Helios Klinikum Berlin und MDC-Forschungsgruppe Molekulare Grundlagen der Herzinsuffizienz, hat Ende 2002 den Ruf der Charité auf eine C3-Professur für das Fachgebiet Innere Medizin mit Schwerpunkt Kardiologie angenommen.

Prof. Dr. Martin Zenke, MDC-Forschungsgruppe Molekular- und Zellbiologie hämatopoietischer Zellen, hat im November 2003 den Ruf auf die C4-Professur für Zellbiologie und als Direktor am Helmholtz-Institut für Biomedizinische Technologie am Universitätsklinikum der Rheinisch-Westfälischen Technischen Hochschule (RWTH) Aachen angenommen.

Awards/Preise 2002–2003

2002

Andreas Bembenek
Young Investigator's Award for the Sentinel Node Congress

Marion Bimmler
Bundesverdienstkreuz

Carmen Birchmeier-Kohler
Gottfried Wilhelm Leibniz-Preis der Deutschen Forschungsgemeinschaft

Jana Bröcker und Janine Conrad
Abiturientenstipendium des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch und der Delbrück'schen Familienstiftung

Jan Hinrich Bräsen
Andreas-Grüntzig-Forschungspreis

Ralf Dechend
Adalbert Buning-Preis

Detlev Ganten
Treviranus-Medaille des Verbandes deutscher Biologen

Brenda Gerull
Oskar-Lapp-Forschungspreis

Michael Gotthardt
Sofja Kovalevskaja-Preis der Alexander von Humboldt-Stiftung

Franziska Jundt und Katrin Hoffmann
Rudolf-Virchow-Forschungspreis der Charité

Dominik N. Müller
New Investigator Award for European Fellows
Dieter-Klaus-Förderpreis für Bluthochdruckforschung

Peter M. Schlag
Theodor-Brugsch-Preis des Vereins der „Freunde und Förderer der Berliner Charité e. V.“

Jung-Bum Shin
1. Preis des MDC- und FMP-Doktorandensymposiums

Roger Y. Tsien
Max-Delbrück-Medaille

Gerd Wallukat
Apherese Innovationspreis 2002 der Hans-und-Marlies Stock-Stiftung

2003

Katharina Baum und Gesine Gunkel
Abiturientenstipendium des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch und der Delbrück'schen Familienstiftung

Boris Engels
1. Preis des MDC- und FMP-Doktorandensymposiums

Detlev Ganten
Chevalier de la Legion d'Honneur

Maik Gollasch
Franz-Volhard-Preis der Gesellschaft für Nephrologie

Ralph Kettritz
Hans-U-Zollinger-Preis der Gesellschaft für Nephrologie

Friedrich Luft
Anthony Raine Award
Björn Folkow Preis

Ronald McKay
Max-Delbrück-Medaille

Peter M. Schlag
Wilhelm-Warner-Preis für Krebsforschung

Clemens A. Schmitt
Kind-Philipp-Preis für Leukämieforschung

Prof. Ronald McKay vom US-Institut für neurologische Erkrankungen und Schlaganfall (Bethesda), Preisträger der Max-Delbrück-Medaille 2003
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Prof. Ronald McKay from the National Institute of Neurological Disorders and Stroke (NINDS), Bethesda/USA, Recipient of the Max-Delbrück-Medal 2003
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Helmholtz Fellows

Helmholtz-Stipendiaten



Helmholtz-Stipendiaten (v. l.): Dr. Stefan Britsch, Dr. Katrin Stade, Dr. Jens Jordan, Dr. Armin Rehm, Dr. Brenda Gerull, Dr. Martin Bergmann, Dr. Daniel Krappmann
Copyright: MDC/Photograph: Uwe Eising

Helmholtz-Fellows (from left): Dr. Stefan Britsch, Dr. Katrin Stade, Dr. Jens Jordan, Dr. Armin Rehm, Dr. Brenda Gerull, Dr. Martin Bergmann, Dr. Daniel Krappmann
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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-Stipendien sind am MDC eingerichtet, um die frühe Unabhängigkeit junger, erfolgversprechender 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 wird ihnen Raum und Infrastruktur sowie ein Sachmittelbudget zur Verfügung gestellt. Die gastgebende Forschungsgruppe garantiert dem Stipendiaten/der Stipendiatin thematische Unabhängigkeit; die selbstständige Einwerbung von Drittmitteln wird erwartet. Das Stipendium wird in der Regel für eine Laufzeit von drei bis fünf Jahren gewährt.

Bewerben können sich Postdoktoranden auf Empfehlung eines Forschungsgruppenleiters aus dem MDC Berlin-Buch beim MDC-Vorstand. Es folgt dann eine Begutachtung in den MDC-Gremien. Im Berichtszeitraum hat das MDC insgesamt 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 by the Graduate Committee. Financial support via PhD fellowships is provided by the MDC.

During 2003, the MDC received approximately 200 applications and accepted 9 applicants into the program.

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) zu 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, vergleichbar mit dem „Master“ oder dem deutschen „Diplom“ besitzen. Die Zulassung zum Internationalen PhD-Programm wird in einem kompetitiven Auswahlverfahren durch den Graduiertenausschuss des MDC ermittelt.

2003 erhielt das MDC rund 200 Bewerbungen, 9 Bewerber sind in das Programm aufgenommen worden. Sie erhalten alle ein Stipendium des MDC.

Congresses and Scientific Meetings

Kongresse und Wissenschaftliche Tagungen

2002

“8th Liposome Research Days Conference”

Max-Delbrück-Centrum für Molekulare Medizin (MDC)
Berlin-Buch
(May 21–24, 2002)

Onkologische Fachtagung

Deutscher Berufsverband für Pflegeberufe
mit Robert-Rössle-Klinik
(June 5–7, 2002)

“Lange Nacht der Wissenschaften” auf dem Campus Berlin-
Buch

(June 15, 2002)

“Wissenschaft macht Schule”

Eine Vortragsreihe für Schüler mit Besuch der interaktiven
Mikroskopausstellung
Max-Delbrück-Centrum für Molekulare Medizin (MDC)
Berlin-Buch,
(June 24–28, 2002)

PhD-Symposium

Max-Delbrück-Centrum für Molekulare Medizin (MDC)
Berlin-Buch
(July 1, 2002)

Laien-Experten-Scenario der AG Bioethik und
Wissenschaftskommunikation des Max-Delbrück-Centrums
für Molekulare Medizin (MDC) Berlin-Buch
(September 26–28; October 19; November 7–9, 2002)

“Ernst Schering Lecture 2002”

Max-Delbrück-Centrum für Molekulare Medizin (MDC)
Berlin-Buch und Schering Forschungsgesellschaft
Ian Wilmut: “Cloning in Biology and Medicine”
(September 26, 2002)

Tumorkonferenz, Helios Klinikum
(September 28, 2002)

“ISGO Conference on Structural Genomics 2002”

International Structural Genomics Organisation (ISGO) und
Max-Delbrück-Centrum für Molekulare Medizin (MDC)
Berlin-Buch
(October 10–13, 2002)

“Berlin Lecture on Molecular Medicine”

Prof. Roger Tsien, Howard Hughes Medical Institute,
University of California San Diego (USCF)
“Imaging Signal Transduction and Protein Sociology”
(November 21, 2002)

“Molecular Biosensors in Neuroscience”

Max-Delbrück-Centrum für Molekulare Medizin (MDC)
Berlin-Buch
(November 21–22, 2002)

2003

“Genomics and proteomics based therapy strategies”

Partnering Workshop between France and Bioregion
Berlin-Brandenburg
(February 17, 2003)

“Anwendung von Imaging und Patch Clamp in den
Zellulären Neurowissenschaften”

Max-Delbrück-Centrum für Molekulare Medizin (MDC)
Berlin-Buch
(May 17, 2003)

“Berlin-Buch Congress of Biotechnology”
 BioTOP Berlin-Brandenburg
 Max Delbrück-Centrum für Molekulare Medizin (MDC)
 Berlin-Buch
 Friedrich-Ebert-Stiftung (FES)
 Berlin-Buch Management GmbH (BBM)
 Bundesverband der Pharmazeutischen Industrie (BPI)
 Berliner Wirtschaftsgespräche
 (June 13–14, 2003)

“Lange Nacht der Wissenschaften” auf dem Campus
 Berlin-Buch
 (June 14, 2003)

13. Symposium zu Leukämie- und Lymphomdiagnostik mit
 Mikroskopie- und Durchflußzytometrie-Kurs
 (“Kieler Symposium”),
 Robert-Rössle-Krebs-Klinik Charité, Campus Berlin-Buch/
 Helios Klinikum Berlin
 (June 18–21, 2003)

PhD Symposium
 Max-Delbrück-Centrum für Molekulare Medizin (MDC)
 Berlin-Buch
 (June 26, 2003)

“Euroglia 2003 – VI. European Meeting on Glial Cell
 Function in Health and Disease”
 Max-Delbrück-Centrum für Molekulare Medizin (MDC)
 Berlin-Buch
 (September 3–6, 2003)

“Common Mechanisms in Development and Cancer”
 Max-Delbrück-Centrum für Molekulare Medizin (MDC)
 Berlin-Buch
 (September 26, 2003)

7. Chirurgische Forschungstage
 Robert-Rössle-Krebs-Klinik, Charité/Helios Klinikum Berlin
 (October 16–18, 2003)

“3rd International Symposium on Obesity and
 Hypertension”
 Franz-Volhard-Clinic for Cardiovascular Diseases, Charité/
 Helios Klinikum Berlin
 (October 23–25, 2003)

2. Bucher Hämatologie-Forum
 Beckman Coulter GmbH
 Robert-Rössle-Krebs-Klinik, Charité/Helios Klinikum Berlin
 (November 7–8, 2003)

“Berlin Lecture on Molecular Medicine”
 Prof. Ronald McKay, National Institute of Neurological
 Disorders and Stroke (NINDS), Bethesda, USA
 “Stem Cells in Science and Medicine”
 (December 11, 2003)

“Bürgerkonferenz zur Stammzellforschung”
 AG Bioethik und Wissenschaftskommunikation des
 Max-Delbrück-Centrums für Molekulare Medizin (MDC)
 Berlin-Buch
 (December 12–14, 2003)

Mit dem frisch gekürten Chemienobelpreisträger Prof. Kurt Wüthrich (re.) von der Eidgenössischen Technischen Hochschule (ETH) Zürich, Schweiz, und Chemienobelpreisträger Prof. Robert Huber vom Max-Planck-Institut für Biochemie, Martinsried, waren gleich zwei Laureaten zur International Conference on Structural Genomics (10.–13. Oktober 2002) nach Berlin-Buch gekommen.

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Nobel Prize Winner Prof. Kurt Wüthrich (right) of the Swiss Federal Institute of Technology (ETH) Zurich and Nobel Prize Winner Prof. Robert Huber from the Max Planck Institute of Biochemistry, Martinsried attended the International Conference on Structural Genomics (Oktober 10–13, 2002) in Berlin-Buch.

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Seminars Seminaire

2002

Speaker	Institute	Titel of Seminar
Ariel	Achtman National Institute for Medical Research, Division of Parasitology, London/UK	B cell responses in murine malaria
Scott V.	Adams Vanderbilt University Nashville/USA	Electrophysiological Investigation of Coupling in the Human Serotonin Transporter
Birgit	Alpen Cornell University, New York/USA	SEREX-analysis in gastric marginalzone B-cell lymphoma of MALT-type
Frank	Baas Academic Medical Center, Neurogenetics Laboratory Amsterdam/NL	Allele specific inhibition, a novel approach to treatment of genetic disease
Konrad	Basler University Zürich/Switzerland	Dpp signalling, cell competition and apoptosis in Drosophila limbs
Uta	Bauer IMT University of Marburg/Gemany	Regulation of gene expression by arginine methylation
Etienne	Baulieu Collège de France, Le Kremlin-Bicêtre, Paris/France	Neurosteroids: A New Function of the Brain. Aging, Memory, Myelination
Yinon	Ben-Neriah The Lautenberg Center for Immunology, Hebrew-University-Hadassah, Jerusalem/Israel	Obstacles en route to degradation: lessons from the NF-kB and Wnt pathways
Stefan	Berry Ruhr-Universität Bochum/Gemany	Biological Electron Transport Systems - Theme and Variations
Paola	Bezzi Université de Lausanne/Switzerland	Prostaglandins mediate a major compo- nent component of astrocyte calcium elevations responsible for receptor-stimu- lated glutamate release
Hans	Bock UT Southwestern Medical Center Dallas/USA	Reelin signaling through lipoprotein receptors - Role of Src family kinases
Ulrich	Bogdahn Neurologische Universitätsklinik Regensburg/Gemany	TGF-Beta and malignant gliomas -from pathogenesis to therapy
Jürgen	Bolz University of Jena, Institute of Zoology/ Gemany	Wiring Molecules for the Assembly of Cortical Circuits

Speaker	Institute	Titel of Seminar
Ralf Brandt	Novartis Pharma AG, Basle/Switzerland	The Reconstituted Genetically Manipulated Mouse Mammary Gland: A novel pre-clinical animal model for cancer research
Armin Braun	Fraunhofer-Institut für Toxikologie, Aerosolforschung und Pharmaforschung Hannover/Germany	Are neurotrophins a suitable target in asthma therapy?
Tom Brismar	Karolinska Hospital, Stockholm/Sweden	Physiology of gliomas
Jean-François Brunet	CNRS UMR 8542, Département de Biologie, Ecole Normale Supérieure, Paris/France	Phox2b - master gene of a neural circuit
Thorsten Burmester	Institute for Zoology, University of Mainz/Germany	Neuroglobin: A respiratory protein of the brain
Selina Chen-Kiang	Cornell University, New York/USA	CDK inhibitors: essential controls of B cell terminal differentiation and tumorigenesis
Stephen Cohen	EMBL Heidelberg/Germany	Boundary formation in Drosophila wing development
Rudolf A. Deisz	Charité University Medicine Berlin/Germany	Impaired function of metabotropic GABA receptors in slices from epilepsy surgery
Gunnar Dittmar	Harvard Medical School/ USA	Hub1 - the ubiquitin-like link to cell polarity
Veit Flockerzi	University Saarland-Saarbrücken, Pharmakologie und Toxikologie/Germany	The trp channels, a remarkably functional family
Mikio Furuse	Dept. of Cell Biology, Kyoto University/Japan	The role of claudins in epithelial barrier function
Ali Gorji	University of Muenster/Germany	Spreading depression in human neocortical slices and rat spinal cord tissues
Magdalena Götz	Max Planck Institute for Neurobiology Munich/Germany	Glial cells generate neurons: molecular and cellular mechanisms of neurogenesis
Ingrid Grummt	Molecular Biology, DKFZ Heidelberg/Germany	Regulation of gene expression in eukaryotes: Lessons learned from RNA polymerase I
Francois Guillemot	Institut de Génétique et de Biologie, Moléculaire et Cellulaire C.U. de Strasbourg/France	Proneural genes and cell type specification in the murine telencephalon
Ulrich Hämmerling	Memorial Sloan-Kettering Cancer Center, New York/USA	Vitamin A: new life for an ancient molecule
Christian Harteneck	Free University Berlin, Institute for Pharmacology/Germany	Structure and function of TRP-homologous channels
Jonathan Horsford	Department of Molecular and Medical Genetics, University of Toronto, Ontario/Canada	Chx10 and Mitf have dynamic and opposing functions in mammalian retinal cell fate decisions
Jonathan Howard	Max Planck Institute of Molecular Cell Biology and Genetics, Dresden/Germany	Chemical-to-Mechanical Energy Transduction by the Motor Protein Kinesin
Miho Inoue-Murayama	Gifu University/Japan	Allelic variation of the dog dopamine receptor D4 gene polymorphic region and its relation to behavioral traits
Berend H. Isermann	Blood Research Institute, Blood Center of Southeastern Wisconsin, Milwaukee/USA	Genetically designed mice provide new insights into the function of the thrombomodulin - protein C pathway

Speaker	Institute	Titel of Seminar
Herbert	Jäckle MPI Göttingen/Germany	From egg to embryo: Functional genetics with the model organism <i>Drosophila</i>
Ricarda	Jahnel Free University Berlin/Germany	Biochemical characterisation of the vanilloid receptor 1 (VR1)
Leszek	Kaczmarek Nencki Institute, Warsaw/Poland	c-Fos/AP-1 to matrix metallo-proteinases, a missing link in neuronal plasticity?
Istvan	Kiss Biological Research Centre, Hungarian Academy of Sciences Szeged/Hungary	Apart from nuclear import - the role of importin-alpha in <i>Drosophila</i> oogenesis
Rüdiger	Klein Max-Planck-Institute of Neurobiology Martinsried/Germany	Ephrins and Eph receptors - bidirectional regulators of development and adult plasticity
Jari	Koistinaho University of Kuopio/Finland	p38 MAPK-mediates increased ischemic vulnerability in beta-APP transgenic mice
Tony	Kouzarides Wellcome/CRC Institute, Cambridge University/UK	Histone methylation in transcriptional control
Georg W.	Kreutzberg Max-Planck-Institute for Neurobiology, Martinsried/Germany	Trial & Error in Microglial Research
Kerstin	Kriegelstein University of Goettingen/Germany	TGF - regulators of cell growth and differentiation
Stephan	Kröger University of Mainz/Germany	The synapse-organizing proteoglycan agrin in the developing CNS: expect the unexpected
Ulrich	Kubitscheck Institut for Experimental Physics, University of Bremen/Germany	Insight into the dynamical structure of the cell nucleus by visualization of single molecules and single RNP particles
Ralf	Kuehn Artemis Pharmaceuticals GmbH Cologne/Germany	New technology to generate KO mice
Damien	Kuffler University of Puerto Rico/South America	Promoting regeneration in the adult human PNS and CNS
Luc	Leybaert Gent University/Belgium	Astrocyte-endothelial calcium talk: a signal at the barrier?
Andrew	Matus Friedrich Miescher Institut, Basel/Switzerland	Actin dynamics at synaptic connections in the brain: mechanisms and possible meanings
Petra	May UT Southwestern Medical Center Dallas/USA	Proteolytic processing of LRP mediates regulated release of its intracellular domain
Kevin J.	McElwee Department of Dermatology, Philipp University Marburg/Germany	Alopecia areata in humans and rodents models
Serge	Muyldermans Vrije Universiteit Brussel/Belgium	Camel antibodies as reporter molecules
Teresa	Nicolson Max Planck Institute for Developmental Biology Tübingen/Germany	Swimming in circles: genetic dissection of hearing and balance in zebrafish
Rienk	Offringa Leiden University Medical Center, Netherlands	Role of serine protease inhibitors (serpins) in dendritic cell survival and tumor immune escape
Thomas	Park University of Illinois at Chicago/USA	Unique sensory adaptations of the naked mole-rat
Michal	Pravenec Czech Academy of Science Praha, Institute of Physiology/Czech Republic	Genetic analysis of a complex metabolic syndrome in the spontaneously hypertensive rat

Speaker		Institute	Titel of Seminar
Andreas	Püschel	Westfälische Wilhelms-Universität, Münster/Germany	Signal transduction by receptors for the repulsive axon guidance signal semaphorin 3A: the role of Rho-like GTPases
Nikolaus	Rajewsky	The Rockefeller University, New York/USA	Computational approaches for decoding transcriptional regulation
Hans-Georg	Rammensee	University of Tübingen/Germany	Towards patient-individual tumor peptide selection for vaccination
Ivan	Raska	Academy of Science Praha, Institute of Experimental Medicine/Czech Republic	Searching for active ribosomal genes
Dan	Roden	Vanderbilt University, School of Medicine, Nashville/USA	The Long QT Syndrome
Klaus	Rohr	University of Cologne/Germany	Development of the thyroid gland in zebrafish
Sascha	Sauer	Max-Planck-Institute for Molecular Genetics Berlin/Germany	Genotyping Single Nucleotide Polymorphisms by MALDI Mass Spectrometry
Mathias	Seeliger	University Eye Hospital Tuebingen/Germany	Analysis of retinal physiology based on animal models of inherited retinal degenerations
Giulio C.	Spagnoli	University of Basel/Switzerland	Induction of CTL against tumor associated antigens in melanoma patients upon immunization with recombinant vaccinia virus
Hans	Stassen	University Hospital Zurich/Switzerland	Genetic diversity - quantification and analysis
David	Stuart	Oxford University, Structural Biology Centre/UK	Towards the Atomic Structure of an Enveloped Virus: Bacteriophage PRD1
Gerd	Sutter	GSF München, Institut für Molekulare Virologie/Germany	Immunogenicity and protective capacity of vaccinia virus MVA vectors in animal models for viral disease and cancer
Bernard	Swynghedauw	INSERM Paris/France	Cardiac remodeling. An evolutionary perspective
Dawn	Teare	University of Sheffield, Mathematical Modelling and Genetic Epidemiology Group/UK	Sampling properties of summary measures of Linkage Disequilibrium
Roger	Tsien	Howard Hughes Medical Institute, University of California San Diego/USA	Imaging Signal Transduction and Protein Sociology
André	Uitterlinden	Erasmus University Rotterdam, Department of Internal Medicine/Netherlands	Genetics of osteoporosis
Gunter	Weiss	MPI für Molekulare Anthropologie, Leipzig/Germany	Analyses of primate Xq 13.3 data- from populations to substitution models
Ian	Wilmot	Roslin Institute, Department of Gene Expression and Development, Edinburgh/Scotland	Cloning in Biology and Medicine
Jürgen	Winkler	Neurologische Universitätsklinik Regensburg/Germany	Functional recovery after hippocampal and cholinergic deafferentation
Lode	Wyns	Freie Universität Brüssel/Belgium	Arsenate reductase, a phosphotyrosine-phosphatase drafted for redox duty
Alpha	Yap	University of Queensland, Brisbane/Australia	Cell-cell recognition: E-cadherin signaling to the actin cytoskeleton
Peter	Zahradka	St. Boniface General Hospital Research Centre, Winnipeg/Canada	Novel targets for antirestenosis intervention

2003

Speaker	Institute	Titel of Seminar
Sylvia	Arber Universität Basel	Molecular pathways controlling the assembly of the spinal monosynaptic reflex circuit
Bernd	Arnold DKFZ Heidelberg/Germany	Tolerance induction by non-professional antigen presenting cells
Uri	Ashery Dept. of Neurobiochemistry Tel-Aviv University/Israel	Molecular mechanisms of synaptic transmission
Mathias	Bähr University of Göttingen, Neurology/Germany	Neuroprotection - models, mechanisms, perspectives
Anne	Baron-van Evercooren CHU Pitié-Salpêtrière, Paris/France	Neural progenitors and CNS myelin repair
Konrad	Basler Dept. of Structural Biology, Stanford University School of Medicine/USA	Growth and patterning in Drosophila limb development
Peter B.	Becker Ludwig-Maximilians University Munich/Germany	Targeting of the dosage compensation complex to the male X chromosome in Drosophila
Jürgen	Behrens Nikolaus Fiebiger Center for Molecular Medicine Erlangen/Germany	Control of Wnt Signaling in Tumorigenesis
Philippe	Bertolino Intern. Agency for Research on Cancer, Lyon, France	Developmental and tumor studies using Men1 knock-out mouse models
Axel	Borst MPI Martinsried/Germany	Neural circuits in the cockpit of the fly
Thomas	Braun University of Halle, Inst. of Physiological Chemistry/Germany	Plasticity of myogenic differentiation: Muscle stem cells in regeneration and development of cardiac and skeletal muscle
Jorge	Caamano MRC Centre for Immune Regulation University of Birmingham	Regulation of secondary lymphoid tissue organogenesis by the NF-kB proteins.
Peter	Carmeliet University of Leuven and Center for Transgene Technology & Gene Therapy/Belgium	Genetics in the mouse, humans and zebrafish to dissect vascular development and disease
Patrick	Carroll University of Montpellier/France	New perspectives on reelin signalling in neuronal migration
Elena	Cattaneo University of Milano/Italy	The huntingtin syndromes
Hans	Clevers Dept. Immunology, University Hospital Utrecht/Netherlands	Sculpting the gut
Pam	Cowin Dept. of Cell Biology, New York University Medical Center/USA	Catenins in the control of mammary and epidermal proliferation and cell fate
Monica	di Luca University of Milano/Italy	Structural organization of the excitatory synapse: role of MAGUK in the Post-synaptic Density
Martin	Eilers Philipps University Marburg/Germany	Mechanisms of Transcriptional Regulation by Myc
Peter	Ekblom Cell and Molecular Biology, Lund University, Lund/Sweden	Role of laminin-1 for epithelial development
Riccardo	Fodde Dept. of Human and Clinical Genetics, Leiden University Medical Center/Netherlands	Stem cell differentiation, signal transduction and genetic instability in colorectal cancer

Speaker		Institute	Titel of Seminar
Stephan	Frank	Dept. of Neuropathology, University Bonn/Germany	Mitochondria during Apoptosis: Divide and Perish ...
Clemens Martin	Franz	Ludwig Institute for Cancer Research, London, UK	Association of p120 catenin with microtubules and the centrosome
Jonas	Frisen	Karolinska Institute Stockholm/Sweden	Generation of neurons from stem cells in the adult brain
Michael	Frotscher	University of Freiburg/Germany	Reelin controls neuronal migration by acting on the radial glial scaffold
Craig	Garner	Stanford University/USA	Cellular mechanisms of CNS synaptogenesis
Max	Gassmann	University of Zürich/Switzerland	Constitutive, HIF-1-independent erythropoietin gene expression: how transgenic mice cope with a hematocrit of 80%
Christo	Goridis	Ecole Normale Supérieure Paris	From the control of neurogenesis to autonomic disorders: the role of the Phox2 transcription factors
Rudolf	Grosschedl	Institute for Biochemistry and Genetics/ University of Munich/Germany	Wnt-dependent and Wnt-independent regulation of organogenesis by LEF1
Christian	Haass	University of Munich	Intramembraneous Proteolysis and Alzheimer's Disease
Alison K.	Hall	Cleveland University/USA	Sensory neurons in development and injury: the role of activin
Matthias	Hammerschmidt	Max Planck Institute for Immunobiology, Freiburg/Germany	BMPs and partners during embryogenesis and organogenesis of the zebrafish
Bernd	Heimrich	Charité University Medicine Berlin, Institute of Anatomy/Germany	Regulators of hippocampal development
Carl-Philipp	Heisenberg	Max Planck Institute for Molecular Cell Biology and Genetics Dresden/Germany	Molecular and cellular control of cell migration during zebrafish gastrulation
Joachim	Herz	University of Texas, Southwestern Medical Center/USA	Mechanisms of signaling by ApoE receptors in the brain and vascular wall
Wolfgang	Holthöner	University of Vienna, Division of General Dermatology, Vienna/Austria	Fibroblast growth factor-2 induces Lef/Tcf-dependent transcription in human endothelial cells
Steve	Hunt	University College London	Substance P in Depression, Addiction and Pain
Beat	Imhof	Department of Pathology, Centre Medical Universitaire Geneva/Switzerland	Analysis of adhesion molecules in cell migration
Rodney	Kellems	University of Texas, Dept. of Biochemistry/USA	Autoantibodies against the Angiotensin Receptor in Preeclampsia
Rolf	Kemler	Department of Molecular Embryology/ Max-Planck Institute of Immunobiology Freiburg/Germany	β -Catenin controls cell fate in mouse development
Susan	Kentner	HGF office Brussels/Belgium	6th European Framework Programme, Contract and Budget Issues, Project Management, Proposals and Results from the 1st Call
Ralph	Kettritz	HELIOS Klinikum Berlin/Charité, Franz Volhard Clinic, Nephrology and Hypertension/Germany	Mechanisms of ANCA-associated Vasculitis

Speaker	Institute	Titel of Seminar	
Christian	Klämbt	University of Muenster/Germany	Genetic Analysis of Neuron-Glia Interaction at the CNS Midline of Drosophila
Wilfried	Kraus	HGF office Brussels/Belgium	6th European Framework Programme. Contract and Budget Issues, Project Management, Proposals and Results from the 1st Call
Michaela	Kuhn	University of Muenster, Institute of Pharmacology and Toxicology/Germany	Cell-restricted deletion of the guanylyl cyclase-A receptor in mice unravels the various physiological functions of atrial natriuretic peptide (ANP)
Erich	Lanka	Max-Planck-Institut für Molekulare Genetik, Berlin	The role of pro-telomerase in the replication of linear DNA
Urban	Lendahl	Karolinska Institute, Stockholm/Sweden	Notch signaling in development and disease
Clotilde	Levecque	Institute Pasteur de Lille, France	Genetic determinants of Parkinson's disease
James K.	Liao	Harvard Medical School, Vascular Medicine Research/USA	Novel Nongenomic Actions of Steroid Hormones on the Vascular Wall
Joachim	Luebke	Research Center Juelich/Germany	Morphological and functional aspects of excitatory synaptic transmission in the barrel cortex
Bernhard	Lüscher	RWTH Aachen, Institut for Biochemistry and Molecular Biology/Germany	The Myc/Max/Mad network: transcriptional control of cell behavior
Lamberto	Maffei	Istituto di Neuroscienze Pisa/Italy	Molecular basis of plasticity in the mammalian visual cortex
Stefanie	Mandl	Stanford University, School of Medicine/USA	Understanding immune cell trafficking via in vivo bioluminescence imaging
Roland	Martin	NIH Bethesda/USA	Novel Findings on the Pathogenesis of Multiple Sclerosis
Evelyne	May	Centre d Energie Atomique Paris/France	Recent data on in vitro and in vivo p53-dependent transcriptional activity
Frank	Mc Cormick	University of California, San Francisco/USA	Cancer Therapy based on Ras and p53
John	McGiff	New York Medical College Valhalla, New York/USA	Renal COX-2, Cytokines, and 20-HETE: Tubular and Vascular Mechanisms
Ronald	McKay	National Institute of Neurological Disorders and Stroke (NINDS), Bethesda/USA	Stem Cells in Science and Medicine
Barbara	Munz	Stanford University Medical Center Stanford/USA	Differential gene expression in myogenic differentiation
Erwin	Neher	University of Goettingen	Dissecting Short Term Synaptic Plasticity at the Calyx of Held
Scott	Ness	Molecular Genetics & Microbiology University of New Mexico/USA	Complexities of gene regulation by Myb transcription factors
Genevieve	Nguyen	INSERM U489, Paris/France	The renin receptor - A new partner in the renin-angiotensin system
Christof	Niehrs	DKFZ Heidelberg/Germany	Molecular mechanism of embryonic head induction
Christiane	Nüsslein-Volhard	Max Planck Institute for Developmental Biology Tübingen/Germany	Cell migration in zebrafish
Stefan	Offermanns	University of Heidelberg/Germany	GPCRs and plexins in neural development and function

Speaker	Institute	Titel of Seminar
Yoshiaki	Ohkuma Osaka University, Laboratory of Cellular Biolog/Japan	Physical and functional links between human mediator complexes and general transcription machinery
Christopher	Payne University Zürich/Switzerland	Motor Proteins, Endomembranes and Nuclear Dynamics During Mammalian Fertilization
Vladimir	Pekarik University of Zürich/Switzerland	RNA interference in ovo to study axon guidance
Michel	Pletschette European Commission Research International Cooperation, Brussels/Belgium	Ethical Issues in clinical research and trials in developing countries
Lincoln R.	Potter University of Minnesota, USA	Regulation of and Signaling Through Cyclase-Linked Natriuretic Peptide Receptors
Klaus T.	Preissner Justus Liebig University Giessen, Institute for Biochemistry/Germany	Trick and Treat: New Endogenous and Exogenous Anti-inflammatory Molecules
Nikolaus	Rajewsky NYU, New York/USA	Computational Decoding of Gene Regula- tory Networks in Multicellular Organisms
Christoph	Redecker Friedrich-Schiller-Universität Jena, Neurology/Germany	Functional consequences of focal lesions in the developing and adult brain
Louis	Reichardt Howard Hughes Medical Institute, Dept. of Physiology, University of California San Francisco/USA	Catenin functions in synapse formation and function
Diethelm	Richter University of Göttingen/Germany	Serotonergic Modulation of the Respiratory Center
Frank	Rosenbauer Harvard Medical School, Beth Israel Deaconess Medical Center, Boston/USA	Stem Cells, Progenitors, and Malignancies: Acute Myeloid Leukemia Induced by Reduction of Transcription Factor PU.1 in Mice
Patricia	Ruiz Max-Planck Institute for Molecular Genetics, Berlin/Gemany	Molecular Approaches to Heart Failure
Kathryn	Sandberg Georgetown University, Washington/USA	Translational control of the angiotensin receptor by alternative splicing
Constance	Scharff MPI of Molecular Genetics Berlin/Germany	Insights from bird brains: pathways for learned vocal communication, regulation of adult neurogenesis, and characterization of a "speech" gene
Martina	Schinke Harvard Medical School, Boston/USA	Cardiogenomics: Identifying gene expres- sion profiles in cardiac hypertrophy
Christian A.	Schmidt Ernst-Moritz-Arndt Universität Greifswald, Zentrum für Innere Medizin	Characterization of atypical T-cell- receptor (TCR) rearrangement
Hans	Schöler Germline Development Group University of Pennsylvania Philadelphia/USA	From ES cells to Oocytes: A stepwise Approach to Nuclear Transplantation Therapy
Markus	Schülke Charité University Medicine Berlin, Institute of Pediatrics/Germany	Proteome analysis in patients with mitochondriopathies
Thomas	Schwartz Rockefeller University, New York/USA	Structure of the eukaryotic SRP-receptor: New insight into the complex regulation of protein targeting
Peter	Seeburg University of Heidelberg	Genetic manipulation of synaptic function in the mouse: Probing synaptic plasticity and spatial learning

Speaker		Institute	Titel of Seminar
Atsuko	Sehara-Fujisawa	Kyoto University/Japan	Roles of Metalloprotease-disintergrin (ADAM)s in morphogenesis
Marjeta	Sentjurc	Jozef Stefan Institute, Ljubljana/Slovenia	Electron paramagnetic resonance (EPR) in the investigation of biological processes
Paul	Sharpe	Kings College London, Dental Institute, Dept. of Craniofacial Development/UK	Molecular control of tooth patterning: towards regenerative dentistry
Curt	Sigmund	University of Iowa, Iowa City/USA	Dissecting complex physiological pathways using targeted gene expression
Mikael	Sigvardsson	Department for Stem Cell Biology, Lund/Sweden	Co-ordination of gene expression by helix-loop-helix transcription factors in early B lymphocyte development
Jan A.	Staessen	Laboratory of Hypertension Campus Gasthuisberg Leuven/Belgium	Essential Hypertension
Archontoula	Stoffel	The Rockefeller University, New York/USA	API2/MALT1, NF-kB and p53: a combi-national network that promotes lympho-magenesis
Max	Topp	University of Tübingen/Germany	Immunotherapy strategies for targeting B-cell malignancies
Elmar	Wahle	University of Halle/Germany	Polyadenylation of pre-mRNA: Mecha-nisms of processive elongation and length control
Andrew H.J.	Wang	Institute of Biological Chemistry, Academia Sinica, Taipei/Taiwan	Structural proteomics in Taiwan
Eva	Wardelmann	Institute of Pathology, University Bonn/Germany	Bedeutung von c-kit-Mutationen für das biologische Verhalten Gastrointestinaler Stromatumoren
Bill	Weis	Albers School, University of Seattle/USA	Biochemical and structural analysis of beta-catenin in cell adhesion and Wnt signaling
Heiner	Westphal	NIH, Bethesda/USA	Transcriptional control of early mouse development
Ralph	Willemsen	Erasmus MC University Medical Center Rotterdam/NL	Genetic engineering of T cell specificity
Martina	Witsch-Baumgartner	Institute of Medical Biology and Human Genetics, Innsbruck/Austria	Population Genetics of the Smith-Lemli-Opitz Syndrome
Xia	Yiang	University of Texas, Dept. of Biochemistry/USA	Autoantibodies against the Angiotensin Receptor in Preeclampsia

Structure and Organization



The MDC Berlin-Buch

Das MDC Berlin-Buch

The MDC Berlin-Buch and Clinical Research

Clinical Research

The Max-Delbrück-Center for Molecular Medicine (MDC) Berlin-Buch makes around five million Euros available from its budget for joint research projects conducted with two specialist clinics of the Charité University Medical School and Hospital Berlin/The Helios Teaching Hospitals Berlin – namely, the Franz-Volhard Cardiovascular Clinic (*FVK*) and the Robert-Rössle Cancer Clinic (*RRK*). As part of these joint projects, researchers and scientists of the MDC search for genes and mechanisms that play a role in diseases such as high blood pressure, metabolic disorders, cardiovascular diseases, and cancer. The four clinical directors of the *FVK* and the *RRK* have their own research group at the MDC. As MDC Research Group Leaders, they are automatically members of all MDC committees including the Management Committee.

MDC scientists have jointly reached agreement on three structural foci of the collaboration with their clinical partners:

- **Clinical Cooperation Projects (KKP):** Here, joint research projects are conducted on the topics of cardiovascular and metabolic diseases, cancer research as well as the function and dysfunction of the nervous system.
- **Clinical Research Units (CRU):** The Clinical Research Units do not serve the care of patients, but are intended for the diagnosis and for the scientific examination of patients, which are necessary within the framework of the clinical cooperation projects. They are a distinctive feature in the research structure of a university clinic and constitute the basis of the clinical research.
- **Clinical Training Program (KAP):** The basic idea of the clinical training program is to make the transition into basic research at the MDC easy for doctors with completed clinical training through targeted fostering of junior group leaders and to lay the foundation for further scientific training.

Das MDC Berlin-Buch und die klinische Forschung

Klinische Forschung

Das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch stellt jährlich fünf Millionen Euro aus seinem Budget für Forschungsvorhaben mit den beiden Spezialkliniken der Charité – Universitätsmedizin Berlin/Helios Klinikum Berlin – der Franz-Volhard-Herz-Kreislaufklinik (*FVK*) und der Robert-Rössle-Krebsklinik (*RRK*) zur Verfügung. In gemeinsamen Forschungsprojekten suchen klinische Forscher und Wissenschaftler des MDC nach Genen und Mechanismen, die bei Krankheiten wie Bluthochdruck, Fettstoffwechselstörungen, erblich bedingten Herzvergrößerungen und Krebs eine Rolle spielen. Die vier leitenden Ärzte der *FVK* und der *RRK* haben gleichzeitig eigene Forschungsgruppen am MDC. Als MDC-Forschungsgruppenleiter sind sie automatisch Mitglieder in allen MDC-Gremien einschließlich des Leitungskollegiums.

Wissenschaftlerinnen und Wissenschaftler des MDC haben sich gemeinsam mit ihren klinischen Partnern auf drei strukturelle Schwerpunkte der Zusammenarbeit verständigt:

- **Klinische Kooperationsprojekte (KKP):** Hier werden von Grundlagenwissenschaftlern und Klinikern gemeinsam Forschungsprojekte zu den Themen Herz-Kreislauf- und Stoffwechsel-Erkrankungen, Krebsforschung sowie Funktion und Dysfunktion des Nervensystems bearbeitet.
- **Clinical Research Units (CRU):** Die Clinical Research Units dienen nicht der Krankenversorgung, sondern sind für Diagnose und für wissenschaftliche Untersuchungen von Patientinnen und Patienten gedacht, die im Rahmen der klinischen Kooperationsprojekte notwendig sind. Sie sind eine Besonderheit in der Forschungsstruktur einer Universitätsklinik und sind die Basis für die klinische Forschung.
- **Klinisches Ausbildungsprogramm (KAP):** Grundidee des klinischen Ausbildungsprogramms ist es, durch gezielte Nachwuchsförderung Medizinerinnen und Mediziner mit abgeschlossener klinischer Ausbildung den Über-

The entire funding for research which the MDC provides for the joint projects ("Twinning Grants") of clinicians and basic research scientists is monitored by internal and external experts. The success of this model was evident through the positive results of an independent evaluation of the clinical research groups in the Cardiovascular and Cancer Research Programs that took place in April 2002. As was seen in the evaluation report of the program oriented funding, the value of the MDC's interdisciplinary concept in the various fields of basic and clinical research was confirmed. Specifically, the auditors highly rated the three structural foci (KKP, CRU, and KAP).

A New Partner, The HELIOS Clinics GmbH

As of June 1, 2001, the HELIOS Kliniken GmbH Fulda became the financial provider of the Buch Clinic as well as the two Charité Clinics, the Robert Rössle Cancer Clinic and the Franz Volhard Cardiovascular Clinic. Thus, it is assured that patient care and research can continue in close cooperation with the Charité and private fund providers. The HELIOS Kliniken GmbH has begun with the new construction of a clinic for approximately 200 million Euros in the direct vicinity of the Berlin-Buch Campus which will be completed at the end of 2005. The Buch University Clinics of the Charité, the FVK, and the RRK will move into the new building together with the Buch Clinics Complex with a total of 1,069 beds. Thus, one of the most modern and largest clinics of Berlin will be located in the direct vicinity of the Berlin-Buch Campus. With the founding of the Helios Research Center GmbH through a Shareholder Contract in June 2002 a first formalized partnership with the private operator HELIOS GmbH has been formed on the part of the MDC Berlin-Buch. The task of the company is to support further science and research in the HELIOS group of teaching hospitals, in particular in the sector of clinical studies, and to obtain third party funding for their financing.

gang in die Grundlagenforschung am MDC zu erleichtern und den Grundstein für die wissenschaftliche Weiterbildung zu legen.

Die gesamte Forschungsförderung, die das MDC für die gemeinsamen Projekte („Twinning Grants“) von Klinikern und Gruppen der Grundlagenforschung aufbietet, wird von internen und externen Experten evaluiert. Der Erfolg dieses Modells lässt sich an den positiven Evaluationsergebnissen der Begutachtung der klinischen Arbeitsgruppen im Bereich der Herz-Kreislauf- sowie Krebsforschung im April 2002 belegen. Wie auch bei der Begutachtung im Rahmen der Programmorientierten Förderung wurde das interdisziplinäre Konzept des MDC in den verschiedenen Bereichen der Grundlagenforschung und der klinischen Anwendung voll bestätigt. Dabei haben die Gutachter die drei strukturellen Schwerpunkte KKP, CRU und KAP insgesamt als besonders positiv bewertet.

Ein neuer Partner, die HELIOS Kliniken GmbH

Zum 1. Juni 2001 hat die HELIOS Kliniken GmbH Fulda als Träger das Klinikum Buch sowie die beiden Berlin-Bucher Charité-Kliniken, Robert-Rössle-Krebsklinik und Franz-Volhard-Herz-Kreislaufklinik, übernommen. Künftig ist damit sichergestellt, dass Forschung und Krankenversorgung in enger Kooperation von Charité und privatem Träger erfolgen können. Die HELIOS Kliniken GmbH hat in unmittelbarer Nähe des Campus Berlin-Buch mit dem Neubau einer Klinik für rund 200 Millionen Euro begonnen, der Ende 2005 fertiggestellt sein wird. In das neue Gebäude werden die Bucher Universitätskliniken der Charité, zusammen mit dem Bucher Klinikum mit insgesamt 1069 Betten einziehen. Damit wird sich eine der modernsten und größten Kliniken Berlins direkt neben dem Campus Berlin-Buch befinden. Mit der Gründung der Helios Research Center GmbH über einen Gesellschaftervertrag im Juni 2002 ist eine erste formalisierte Partnerschaft zum privaten Betreiber HELIOS GmbH seitens des MDC Berlin-Buch hergestellt worden. Aufgabe der Gesellschaft ist es, Wissenschaft und Forschung in der HELIOS Kliniken Gruppe, insbesondere im Bereich klinischer Studien, zu unterstützen und Drittmittel zu deren Finanzierung einzuwerben.

Die Robert-Rössle-Krebs-Klinik der Charité/Helios Klinikum Berlin auf dem Campus Berlin-Buch. Ärzte dieser Klinik arbeiten eng mit Wissenschaftlern des MDC Berlin-Buch zusammen.

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The Robert Roessele Cancer Clinic of the Charité/Helios Klinikum Berlin on the Berlin-Buch Campus. Physicians of this clinic work closely together with scientists of the MDC Berlin-Buch.

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Das Experimental and Clinical Research Center Charité/MDC Berlin-Buch (ECRC)

Eine wichtige Schlussfolgerung aus den Begutachtungen des MDC und den Kliniken in Berlin-Buch ist die Verstärkung und der Ausbau von Schnittstellen und institutionellen Verbindungsbrücken zwischen der Grundlagenforschung und der Klinik. Diese Überlegungen haben zum Konzept geführt, die klinischen Forschungsprojekte des MDC und der Kliniken zu einem „Experimental and Clinical Research Center“ Charité/MDC Berlin-Buch (ECRC) zusammenzuführen. Es soll in der 2005/2006 frei werdenden Robert-Rössle-Klinik (Charité) und in einem Gebäude in direkter Verbindung zum HELIOS-Klinikum Berlin-Buch angesiedelt werden. Die Verbindung des Potentials des Forschungsbereichs Gesundheitsforschung der Helmholtz-Gemeinschaft mit der Charité-Universitätsmedizin Berlin und Partnern der Industrie wie Schering, Philips, General Electric und Siemens im Bereich der Entwicklung von NMR-Technologien bietet die Möglichkeit, solch ein Zentrum von nationaler und internationaler Bedeutung zu schaffen.



Die Franz-Volhard-Herz-Kreislauf-Klinik der Charité/Helios Klinikum Berlin auf dem Campus Berlin-Buch. Ärzte dieser Klinik arbeiten eng mit Wissenschaftlern des MDC Berlin-Buch zusammen.

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The Franz Volhard Clinic for Cardiovascular Diseases of the Charité/Helios Klinikum Berlin on the Berlin-Buch Campus.

Physicians of this clinic work closely together with scientists of the MDC Berlin-Buch.

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The Experimental and Clinical Research Center Charité/ MDC Berlin-Buch (ECRC)

The expert examinations of the MDC and the clinics in Berlin-Buch confirmed the necessity of setting up interfaces and institutional links between basic research and the clinic. These suggestions lead to the concept of combining the clinical research projects of the MDC and the clinics in an "Experimental and Clinical Research Center" Charité/MDC Berlin-Buch (ECRC). The ECRC will be located in the current Robert-Rössle-Clinic (Charité) building which will become available in 2005/2006 and in a building directly linked to the HELIOS Clinics Berlin-Buch. The linking of the potential of the research sector Health Research of the Helmholtz Association with the Charité University Medicine Berlin and partners in industry, such as Schering, Philips, General Electric, and Siemens, in the sector of the development of NMR Technologies offers the possibility of creating a center of national and international significance.

The MDC Berlin-Buch and the Helmholtz Association

The Helmholtz Association

The Helmholtz Association (HGF) 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 GSF Research Center for the Environment and Health GmbH, the DKFZ Cancer Research Center, Heidelberg, the GBF Society for Biotechnological Research GmbH, and the MDC. The health research centers work in various areas of medical science, in

Das MDC Berlin-Buch und die Helmholtz-Gemeinschaft

Die Helmholtz-Gemeinschaft

Die Helmholtz-Gemeinschaft (HGF) ist mit ihren 15 Forschungszentren und einem Jahresbudget von rund 2,1 Milliarden Euro 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 GSF Forschungszentrum für Umwelt und Gesundheit GmbH, das DKFZ Krebsforschungszentrum Heidelberg, 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 HGF reformiert und das neue Instrument der programmorientierten Förderung (PROF) 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 mit diesen einem Wettbewerb innerhalb der Helmholtz-Gemeinschaft. Die Programme und Beiträge der einzelnen Zentren werden von internationalen Experten begutachtet. Die daraus resultierende Förderempfehlung des HGF Senats ist Grundlage für die Finanzierung der Zentren durch die Zuwendungsgeber. Inner-

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 HGF 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 HGF 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 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. It resulted in the program sections Cancer and Neurosciences being described as scientifically outstanding. In the Cardiovascular Program, great productivity was attested in the MDC research groups and their work was assessed as competitive and above the international average. This high estimation of the research work of the MDC Berlin-Buch was also confirmed overall by the ISI-Analysis in the National Citation Report 2001. According to this analysis, the MDC Berlin-Buch takes one of the top positions among the German Institutes conducting molecular medical research.

Laboratory for Medical Genome Research

Already before the HGF 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 Institute for Molecular Pharmacology (FMP) and, thus, 62.5% of the costs will be financed by the EFRE. The new laboratory building will be constructed at the end of the main axis of the Campus grounds in the direct vicinity of the FMP

halb 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 (PROF) 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 Stoffwechsel-Erkrankungen (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 rein strategische Programmbegutachtung. Als Ergebnis wurden die Programme Krebs und Neurowissenschaften als wissenschaftlich herausragend bezeichnet. Auch im Herz-Kreislaufprogramm wurde den MDC-Forschungsgruppen eine große Produk-

Grundsteinlegung für das Labor für Medizinische Genomforschung auf dem Campus Berlin-Buch am 24. Juni 2003. Bauherren sind das MDC Berlin-Buch und das Forschungsinstitut für Molekulare Pharmakologie (FMP). Die Maurerkelle schwingen (v. l.) Prof. Walter Rosenthal (FMP), der Regierende Bürgermeister von Berlin, Klaus Wowereit, der MDC-Kuratoriumsvorsitzende, Ministerialdirektor Reinhard Junker vom Bundesforschungsministerium und Prof. Detlev Ganten, MDC-Stiftungsvorstand.

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Laying the foundation stone for the Laboratory for Medical Genomics on the Berlin-Buch Campus on June 24, 2003. It is jointly erected by the MDC Berlin-Buch and the Forschungsinstitut für Molekulare Pharmakologie (FMP). Swinging the trowel are (from left): Prof. Walter Rosenthal (FMP), the Governing Mayor of Berlin, Klaus Wowereit, the chairman of MDC's Board of Trustees, Ministerialdirektor Reinhard Junker from the Federal Research Ministry, and Detlev Ganten, M.D., Ph.D, MDC's Scientific Director.

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Der Campus Berlin-Buch, 2003
The Campus Berlin-Buch, 2003

and the Walter-Friedrich House of the MDC. The completion is planned for the spring of 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 Forschungsverbund Berlin 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 40 companies are located on the Campus of which around 30 are in the biotechnology sector. The companies employ a total of around 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 Lab (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

aktivität attestiert und ihre Arbeit als kompetitiv und über dem internationalen Durchschnitt bewertet. Diese hohe Einschätzung der Forschungsarbeiten des MDC Berlin-Buch wird auch insgesamt durch die Analyse des Institute for Scientific Information (ISI) in Philadelphia/USA im National Citation Report 2001 bestätigt. Nach dieser Analyse nimmt das MDC Berlin-Buch unter den deutschen Instituten, die molekulare medizinische Forschung betreiben, einen der vordersten Plätze ein.

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 von MDC Berlin-Buch und dem Forschungsinstitut für Molekulare Pharmakologie (FMP), dabei werden 62,5 Prozent der Kosten aus dem Europäischen Fonds für Regionalentwicklung (EFRE) finanziert. Der Laborneubau wird am Ende der Hauptachse des Campusgeländes in direkter Nachbarschaft zum FMP und dem Walter-Friedrich-Haus des MDC errichtet. Die Fertigstellung ist für Frühjahr 2005 vorgesehen. Mit dem Neubau des Labors für Medizinische Genomforschung werden die räumlichen Kapazitäten ausgebaut und damit die Voraussetzungen geschaffen, neue Fragestellungen in der Genomforschung zu bearbeiten

Das MDC Berlin-Buch und der Campus-Berlin-Buch

BBB Management GmbH mit Innovations- und Gründerzentrum

Die BBB Management GmbH (BBB) wurde 1995 durch das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch gegründet. Mitgesellschafter sind der Forschungs-

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 people 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.



Bundesjustizministerin Brigitte Zypries lässt sich von Dr. Ulrich Scheller im Gläsernen Labor in die gentechnische Arbeitswelt einführen. (22. Juli 2003)

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Introduction into the world of genetic engineering - the Federal Minister/Secretary of Justice, Brigitte Zypries and Dr. Ulrich Scheller in the Life Science Learning Lab. (July, 22, 2003)

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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

verbund Berlin (20%) sowie die Schering AG (20%). Die BBB ist die Entwicklungs- und Betreibergesellschaft des Campus Berlin-Buch. Auf insgesamt ca. 26.000 m² Nutzfläche stehen, insbesondere im geförderten Innovations- und Gründerzentrum (IGZ), zu attraktiven Konditionen Gewerbeeinheiten zur Verfügung, in den kleine und mittelständige Unternehmen forschen und produzieren. Es gelang 2003, mehrere neue Unternehmen, darunter auch drei Neugründungen, auf dem Campus anzusiedeln. Gegenwärtig sind auf dem Campus rund 40 Unternehmen angesiedelt, davon sind circa 30 Biotechnologieunternehmen. Die Firmen beschäftigen insgesamt circa 500 Mitarbeiter.

2003 hat die BBB Management GmbH Campus Berlin-Buch ein mit rund 16 Millionen Euro errichtetes Labor- und Bioinformatikgebäude eröffnet und damit gleichzeitig das fünfjährige Bestehen des IGZ gefeiert. Der Neubau hat eine vermietbare Fläche von rund 8.000 m² und ist Sitz verschiedener Unternehmen. Mit dem Neubau wurde der Ausbau des IGZ abgeschlossen. Damit verfügt der Campus über eines der größten branchenspezifischen Gründerzentren in Deutschland.

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. 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. Allein im Jahre 2003 nutzten mehr als 7.100 Besucher die umfassenden Angebote zur Fort- und Weiterbildung inklusive Gentechnik, Zellbiologie und Proteinanalysen. In 2003 wurde gemeinsam mit der TÜV Akademie Berlin ein neues Qualifizierungsangebot für Laborkräfte angeboten – ein zehnwöchiger zertifizierter Weiterbildungskurs zur Fachkraft für Molekularbiologie. Im Herbst 2003 eröffnete die BBB Management GmbH in den Räumen des Gläsernen Labors ein Informationsbüro, das gleichzeitig Besuchertreff ist. Es zeigt eine Campusausstellung, und ist Ausgangspunkt für Campus-Führungen.

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 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 Zwillingstudien, die präklinische und klinische Prüfung und Entwicklung von neuen, lipidbasierten Darrei-

ingredient-screening. The funding for the InnoRegio Berlin-Buch through 2005 amounts to 5.2 million Euro.

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.



Das Forschungsinstitut für Molekulare Pharmakologie (FMP) auf dem Campus Berlin-Buch, ein enger Kooperationspartner des MDC Berlin-Buch.
Copyright: Helios Klinikum Berlin/Photograph: Thomas Oberländer
The Institute for Molecular Pharmacology (FMP) at the Berlin-Buch Campus, a close collaborator of the MDC-Berlin-Buch.
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chungsform (drug delivery system) für Krebsmedikamente Taxol, sowie das Protein- und Wirkstoffscreening. Insgesamt wird InnoRegio Berlin-Buch bis zum Jahr 2005 mit 5,2 Millionen Euro gefördert.

Forschungsinstitut für Molekulare Pharmakologie (FMP)

Das Forschungsinstitut für Molekulare Pharmakologie (FMP) betreibt Grundlagenforschung zur Identifizierung und Nutzbarmachung potentieller Zielstrukturen für Pharmaka. Durch die räumliche Nähe wurde die bereits bestehende Zusammenarbeit zwischen MDC und FMP erheblich intensiviert. 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, hat das FMP die funktionelle und strukturelle Charakterisierung von Proteinen zum Ziel, sowie die Entwicklung von Strategien zu ihrer pharmakologischen Beeinflussung.

Auch auf der organisatorischen Ebene gibt es eine Verflechtung beider Forschungseinrichtungen. So werden Großgeräte gemeinsam genutzt und gemeinsam betrieben. Gastwissenschaftlerverträge ermöglichen es 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 finanzieren und gestalten MDC und FMP gemeinsam Veranstaltungen für Doktoranden.

Organizational Structure

The Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch is a foundation under public law of the State of Berlin with the purpose of pursuing medical research at the molecular and cellular levels and implementing its clinical application. As laid down in the bylaws, the bodies of the Foundation are comprised of the following two boards:

- The Board of Trustees
- The Executive Board of the Foundation

The Board of Trustees

The Board of Trustees is the Supervisory Committee of the MDC and monitors the legality, appropriateness, and economic viability of the management of the Foundation's business. It decides on the general objectives of the research and important research policy and financial matters. Up until September 2002, Wolf-Michael Catenhusen, parliamentary Permanent Secretary of the Federal Ministry for Education and Research (*BMBF*), held the office. His successor as Chairman is Reinhard Junker, Head of the Department of Health, Biosciences and Continuity in the *BMBF*. In 2004, a new appointment of the Vice Chairman will be announced following the departure of Dr. Peer Pasternack, Permanent Secretary of the Berliner Senate Administration for Science, Research, and Culture.

Members of the Board of Trustees
 Head of Dept. Reinhard Junker, Federal Ministry for Education and Research Berlin (Chairman)
 (To be named), Senate's Administration for Science, Research and Culture, Berlin (Vice Chairperson)
 Prof. Günter Breithardt, Münster University
 Prof. Hans R. Brunner, Centre Hospitalier Universitaire Vaudois, Lausanne/Switzerland
 Prof. Manfred Dietel, Charité University Medicine, Berlin
 Prof. Bärbel Friedrich, Humboldt University, Berlin
 Prof. Falko Herrmann, Greifswald University
 Prof. Dr. Reinhard Jahn, Max Planck Institute for Biophysical Chemistry, Göttingen

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. Wie im Stiftungsgesetz des MDC verankert, sind die Organe der Stiftung:

- das Kuratorium
- der Stiftungsvorstand

Das Kuratorium

Das Kuratorium ist das Aufsichtsgremium des MDC und überwacht die Rechtmäßigkeit, Zweckmäßigkeit und Wirtschaftlichkeit der Führung des Stiftungsgeschäftes. Es entscheidet über die allgemeinen Forschungsziele und wichtigen forschungspolitischen und finanziellen Angelegenheiten der Stiftung. Bis September 2002 hatte der Parlamentarische Staatssekretär Wolf-Michael Catenhusen vom Bundesministerium für Bildung und Forschung (BMBF) das Amt inne. Sein Nachfolger als Vorsitzender ist Ministerialdirektor Reinhard Junker, Abteilungsleiter Gesundheit, Biowissenschaften, Nachhaltigkeit im BMBF. Der stellvertretende Vorsitz wird nach Ausscheiden von Staatssekretär Dr. Peer Pasternack von der Berliner Senatsverwaltung für Wissenschaft, Forschung und Kultur im Jahre 2004 berufen.

Mitglieder des Kuratoriums
 MinDir Reinhard Junker, Bundesministerium für Bildung und Forschung, Berlin (Vorsitz)
 N.N., Senatsverwaltung für Wissenschaft, Forschung und Kultur, Berlin (stellv. Vorsitz)
 Prof. Günter Breithardt, Universität Münster
 Prof. Hans R. Brunner, Centre Hospitalier Universitaire Vaudois, Lausanne/Schweiz
 Prof. Manfred Dietel, Charité-Universitätsmedizin Berlin
 Prof. Bärbel Friedrich, Humboldt-Universität zu Berlin
 Prof. Falko Herrmann, Universität Greifswald
 Prof. Dr. Reinhard Jahn, Max-Planck-Institut für biophysikalische Chemie, Göttingen



Das Max-Delbrück-Haus des MDC.
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 MDC's Max Delbrück House
 Copyright: MDC/Photographer: Uwe Eising

Department Head Oskar-Peter Kaye, Federal Ministry for Education and Research Bonn
 Senator Dr. Heidi Knake-Werner, Senate's Administration for Health, Social Matters and Consumer Protection, Berlin
 Permanent Secretary Dr. Hermann Schulte-Sasse, of the Senate's Administration for Health, Social Matters and Consumer Protection, Berlin
 Prof. Dr. Dieter Lenzen, Free University of Berlin
 Dr. Gary R. Lewin, MDC, Berlin-Buch
 Prof. Jürgen Mlynek, Humboldt University, Berlin
 Prof. Annemarie Poustka, German Cancer Research Center (DKFZ), Heidelberg
 Senior Government Officer Markus Siebels, Federal Ministry of Finance, Berlin
 Dr. Thomas Sommer, MDC, Berlin-Buch
 Dr. Albert Statz, Federal Ministry for Health, Bonn
 Prof. Dr. Axel Ullrich, Max Planck Institute for Biochemistry, Martinsried
 Prof. Dr. Kurt von Figura, Göttingen University

The Scientific Committee

The Scientific Committee of the Board of Trustees was directed by Prof. Volker ter Meulen until 2003. The Scientific Committee prepares the decisions of the Board of Trustees in scientific questions. It bears the responsibility for the continual evaluation of results of the MDC's research work through scientific examination. In addition to the scientific members of the Board of Trustees, external scientific specialists are also members of the Scientific Committee.

Ministerialrat Oskar-Peter Kaye, Bundesministerium für Bildung und Forschung, Bonn
 Senatorin Dr. Heidi Knake-Werner, Senatsverwaltung für Gesundheit, Soziales und Verbraucherschutz, Berlin
 Staatssekretär Dr. Hermann Schulte-Sasse, Senatsverwaltung für Gesundheit, Soziales und Verbraucherschutz, Berlin
 Prof. Dr. Dieter Lenzen, Freie Universität Berlin
 Dr. Gary R. Lewin, MDC, Berlin-Buch
 Prof. Jürgen Mlynek, Humboldt-Universität zu Berlin
 Prof. Annemarie Poustka, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg
 Regierungsdirektor Markus Siebels, Bundesministerium für Finanzen, Berlin
 Dr. Thomas Sommer, MDC, Berlin-Buch
 Dr. Albert Statz, Bundesministerium für Gesundheit, Bonn
 Prof. Dr. Axel Ullrich, Max-Planck-Institut für Biochemie, Martinsried
 Prof. Dr. Kurt von Figura, Universität Göttingen

Der Wissenschaftliche Ausschuss

Den Wissenschaftlichen Ausschuss des Kuratoriums leitete bis 2003 Prof. Volker ter Meulen. Der Wissenschaftliche Ausschuss bereitet die Entscheidung 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 auch externe Wissenschaftler an.

Members of the Scientific Committee

Prof. Dr. Kurt von Figura, Göttingen University
 Prof. Rudi Balling, Society for Biotechnological Research (GBF), Braunschweig
 Prof. Günter Breithardt, Münster University
 Prof. Hans R. Brunner, Centre Hospitalier Universitaire Vaudois, Lausanne/Switzerland
 Prof. Bärbel Friedrich, Humboldt University Berlin
 Prof. Dr. Roger Goody, Max Planck Institute for Molecular Physiology, Dortmund
 Prof. Falko Herrmann, Greifswald University
 Prof. Dr. Christoph Huber, Mainz University
 Prof. Dr. Wieland Huttner, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden
 Prof. Dr. Reinhard Jahn, Max Planck Institute for Biophysical Chemistry, Göttingen
 Prof. Thomas Meitinger, GSF Research Center for the Environment and Health, Neuherberg
 Prof. Dr. Leena Peltonen, Helsinki University, Finland
 Prof. Annemarie Poustka, German Cancer Research Center, Heidelberg
 Professor Sir George K. Radda, Oxford University, Great Britain
 Prof. Dr. Axel Ullrich, Max Planck Institute for Biochemistry, Martinsried

Mitglieder des Wissenschaftlichen Ausschusses

Prof. Dr. Kurt von Figura, Universität Göttingen
 Prof. Rudi Balling, Gesellschaft für Biotechnologische Forschung (GBF), Braunschweig
 Prof. Günter Breithardt, Universität Münster
 Prof. Hans R. Brunner, Centre Hospitalier Universitaire Vaudois, Lausanne/Schweiz
 Prof. Bärbel Friedrich, Humboldt-Universität zu Berlin
 Prof. Dr. Roger Goody, Max-Planck-Institut für molekulare Physiologie, Dortmund
 Prof. Falko Herrmann, Universität Greifswald
 Prof. Dr. Christoph Huber, Universität Mainz
 Prof. Dr. Wieland Huttner, Max-Planck-Institut für molekulare Zellbiologie und Genetik, Dresden
 Prof. Dr. Reinhard Jahn, Max-Planck-Institut für biophysikalische Chemie, Göttingen
 Prof. Thomas Meitinger, GSF Forschungszentrum für Umwelt und Gesundheit, Neuherberg
 Prof. Dr. Leena Peltonen, Universität Helsinki, Finnland
 Prof. Annemarie Poustka, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg
 Professor Sir George K. Radda, Universität Oxford, Großbritannien
 Prof. Dr. Axel Ullrich, Max-Planck-Institut für Biochemie, Martinsried

The Executive Board of the Foundation

The Executive Board of the Foundation directs the MDC and consists of a Scientific Director, his deputy, and an Administrative Director. Since January 1992, Professor Detlev Ganten has been the Scientific Director of the MDC. His deputy is Professor Walter Birchmeier. Dr. Waltraud Kreutz-Gers took over the administrative direction of the Institute until February 2003. Her successor was Dr. Stefan Schwartze in the summer of 2003.

The Scientific Council

Under the direction of Dr. Martin Lipp, the Scientific Council advises the Executive Board of the Foundation in matters of fundamental scientific importance. In addition to the MDC scientific program coordinators, additional MDC scientific staff members are members of the Scientific Council.

Members of the Scientific Council

Prof. Walter Birchmeier
 Prof. Thomas Blankenstein
 Dr. Kurt Bommert
 Dr. Iduna Fichtner
 Dr. Hannelore Haase
 Prof. Udo Heinemann
 Dr. Uta Höpken
 Prof. Helmut Kettenmann
 Prof. Dr. Gary Lewin
 Dr. Martin Lipp (Chairman)
 Prof. Friedrich Luft
 Dr. Margret Irmgardt Moré
 Dr. Thomas Müller
 Dr. Claus Scheidereit

Der Campus Berlin-Buch
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 The Campus Berlin-Buch
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Der Stiftungsvorstand

Der Stiftungsvorstand leitet das Institut und besteht aus einem wissenschaftlichen Mitglied und einem administrativen Mitglied. Seit Januar 1992 ist Prof. Detlev Ganten wissenschaftlicher Direktor des MDC. Er wird vertreten durch Prof. Walter Birchmeier. Bis Februar 2003 hatte Dr. Waltraud Kreutz-Gers die administrative Leitung des Instituts übernommen. Ihre Nachfolge trat Dr. Stefan Schwartze im Sommer 2003 an.

Prof. Peter Schlag
 Dr. Katrin Stade
 Dr. Ruth Schmidt-Ullrich
 Prof. Wolfgang Uckert (Vice Chairman)
 Dr. Gerd Wallukat

The Staff Council

The Staff Council is primarily involved with decisions at the MDC which concern personnel issues. The chairperson of the Staff Council is Mrs. Marion Bimmler.

Members of the Staff Council
 Marion Bimmler (Chairperson)
 Gabriele Born
 Lutz Else (Vice Chairperson)
 Ingrid Grunewald
 Frank-Peter Kirsch
 Ilse-Maria Knoblich
 Dr. Peter Konzer
 Bernd Lemke (Vice Chairperson)
 Jana Richter
 Christel Westen
 Dr. Reinhard Zeisig

Der Wissenschaftliche Rat

Der Wissenschaftliche Rat berät unter Leitung von Dr. Martin Lipp den Stiftungsvorstand in den Angelegenheiten von grundsätzlicher wissenschaftlicher Bedeutung. Dem Wissenschaftlichen Rat gehören neben den Koordinatoren der Forschungsschwerpunkte weitere wissenschaftliche Mitarbeiter des MDC an.

Mitglieder des Wissenschaftlichen Rates

Prof. Walter Birchmeier
 Prof. Thomas Blankenstein
 Dr. Kurt Bommert
 Dr. Iduna Fichtner
 Dr. Hannelore Haase
 Prof. Udo Heinemann
 Dr. Uta Höpken
 Prof. Helmut Kettenmann
 Prof. Dr. Gary Lewin
 Dr. Martin Lipp (Vorsitz)
 Prof. Friedrich Luft
 Dr. Margret Irmgardt Moré
 Dr. Thomas Müller
 Dr. Claus Scheidereit
 Prof. Peter Schlag
 Dr. Katrin Stade
 Dr. Ruth Schmidt-Ullrich
 Prof. Wolfgang Uckert (stellv. Vorsitz)
 Dr. Gerd Wallukat

Der Personalrat

Der Personalrat ist insbesondere an solchen Entscheidungen des MDC Berlin-Buch beteiligt, welche die personellen und sozialen Belange der Beschäftigten betreffen. Personalratsvorsitzende ist Frau Marion Bimmler.

Mitglieder des Personalrates

Marion Bimmler (Vorsitz)
 Gabriele Born
 Lutz Else (stellv. Vorsitz)
 Ingrid Grunewald
 Frank-Peter Kirsch
 Ilse-Maria Knoblich
 Dr. Peter Konzer
 Bernd Lemke (stellv. Vorsitz)
 Jana Richter
 Christel Westen
 Dr. Reinhard Zeisig

Supporting Divisions

Safety

The task of the MDC Safety Group is to ensure the safety of the MDC employees during their working hours and to prevent occupational accidents and work-related diseases. One of the most important functions of the safety officers is risk assessment, i.e. the identification and assessment of risks during the handling of hazardous substances and biological agents. The Occupational Safety Committee at the MDC consists of the radiation protection officer, the biosafety officer, the employee physician, a representative of the Staff Council, and a safety representative from each laboratory and is directed by the Administrative Director.

Head: Dr. Regina Möhring

Stabsstellen

Sicherheit

Aufgabe der Gruppe Sicherheit ist es, die Mitarbeiter des MDC vor Gefährdungen bei ihren unterschiedlichen Tätigkeiten zu schützen, d.h. Arbeitsunfälle und berufsbedingte Erkrankungen zu verhindern. Eine der wichtigsten Aufgaben der Sicherheitsfachkräfte besteht in der Gefährdungsbeurteilung, d.h. in der Identifizierung und Beurteilung von Risiken während des Umgangs mit gefährlichen Stoffen und biologischen Agenzien. Der Arbeitsschutzausschuss am MDC setzt sich zusammen aus dem Strahlenschutzbevollmächtigten, der Beauftragten für Biologische Sicherheit, der Betriebsärztin, einem Vertreter des Personalrates, einem Sicherheitsbeauftragten aus jeder Arbeitsgruppe und wird vom Administrativen Vorstand geleitet.

Leiterin: Dr. Regina Möhring

Hermann-von-Helmholtz-Haus
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Building and Technology

Façade

The extensive overall renovation measures at the Max-Delbrück House were completed in the first half of 2002 with the renovation of the façade and the roof. The building shell was renewed corresponding to the current requirements regarding protection against heat loss, laboratory operations, and necessary maintenance.

Combined Office and Animal Experiment Building

The construction measure "New construction of an Animal Laboratory and Office Building" for joint use by the MDC and the Institute for Molecular Pharmacology (FMP) was authorized in the year 2000. The five-story new building (Hermann-von-Helmholtz-House) consists of a unit for animal experiments and an office unit. The administration and some research groups of the MDC moved into this building in the spring of 2003. The Animal Experiment Building will be completed in May 2004 and be occupied by the end of the year 2004.

Laboratory for Medical Genome Research

The MDC and FMP are establishing a joint Laboratory building for the Medical Genome Research. The construction work commenced at the end of 2003 and completion is scheduled for 2005.

Head: Grit Kuhlmann

Das Max Delbrück Communications Center (MDC.C) (li.) und das Hermann-von-Helmholtz-Haus des MDC. Letzteres war am 6. Mai 2003 eingeweiht worden.

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The Max Delbrück Communications Center (MDC.C) (left) and the Hermann-von-Helmholtz-House of the MDC, the latter having been inaugurated on May 6, 2003.

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Bau und Technik

Fassade

Die umfangreichen Gesamt-sanierungsmaßnahmen am Max-Delbrück-Haus sind in der ersten Hälfte des Jahres 2002 mit der Sanierung der Fassade und des Daches abgeschlossen worden. Die Gebäudehülle wurde entsprechend der heutigen Anforderungen an Wärmeschutz, Laborbetrieb und notwendiger Instandhaltung erneuert.

Kombiniertes Tierlabor- und Theoriegebäude

Die Baumaßnahme „Neuerrichtung eines Tierlabor- und Theoriegebäudes“ für die gemeinsame Nutzung durch das MDC und das Forschungsinstitut für Molekulare Pharmakologie (FMP) wurde im Jahr 2000 genehmigt. Der fünfstöckige Neubau (Hermann-von-Helmholtz-Haus) besteht aus einer tierexperimentellen und aus einer theoretischen Einheit. Es wurde im Frühjahr 2003 von der Verwaltung und einigen Forschungsgruppen des MDCs bezogen. Das tierexperimentelle Gebäude wird im Mai 2004 baulich fertiggestellt und bis Ende des Jahres 2004 bezogen sein.

Labor für Medizinische Genomforschung

Das MDC und FMP errichten gemeinsam ein Laborgebäude für die Medizinische Genomforschung. Die Bauarbeiten wurden Ende 2003 begonnen, und werden voraussichtlich planungsgemäß im Jahr 2005 abgeschlossen.

Leiterin: Grit Kuhlmann

Revision und Recht – Patente und Lizenzen

Innenrevision

Aufgabe der Revision ist es zu überprüfen, ob Gesetze, Verordnungen, Richtlinien und Weisungen des Stiftungsvorstandes eingehalten und ob die zugewiesenen öffentlichen Mittel zweckentsprechend, sparsam und wirtschaftlich eingesetzt werden. Dazu werden aufgrund eines vom Vorstand bestätigten jährlichen Revisionsplanes Ordnungsmäßigkeits- und Organisationsprüfungen vorgenommen. Zur Aufgabe der Innenrevision gehört auch die Zusammenarbeit mit externen Prüfern und die Erarbeitung von Stellungnahmen zu den Berichten dieser externen Prüfer.

Rechtsangelegenheiten

Schwerpunkt der Arbeit in diesem Bereich ist die Vorbereitung und der Abschluss von Forschungsverträgen mit Industriepartnern und Kooperationsverträgen mit anderen Forschungseinrichtungen.

Patente und Lizenzen

In diesem Bereich geht es vor allem darum, als unmittelbarer Ansprechpartner für alle Wissenschaftlerinnen und Wissenschaftler in Fragen des Technologietransfers zur Verfügung zu stehen. Hier erfolgt eine enge Zusammenarbeit mit der aus der Life-Science-Stiftung hervorgegangenen Verwertungsgesellschaft Ascension GmbH.

Leiterin: Anja Frahn

Auditing, Legal Affairs, Patents, and Licenses

Internal Audit

The goal of the internal audit is to examine whether laws, official regulations, guidelines, and instructions from the Foundation Board of the MDC are being observed and whether the allocated public funds are being used appropriately, sparingly, and economically. For this reason, audits of the compliance with regulations and the organization are undertaken on the basis of an annual audit plan confirmed by the Foundation Board. The collaboration with external auditors and the drawing up of statements in respect to the reports of these external auditors is also included in the task of the internal audit.

Legal Matters

The main focus of the work in this sector is the preparation and finalizing of research contracts with partners from industry and cooperation contracts with other research establishments.

Patents and Licenses

This sector involves serving as the direct contact for all scientists in questions of technology transfer. Here, we maintain a close collaboration with the intellectual property management company Ascenion GmbH, which emerged from the Life Science Trust.

Head: Anja Frahn

Technology Transfer

In the years 2002 and 2003, the sector of technology transfer at the MDC underwent an extensive re-organization. An external partner, Ascenion GmbH, began managing the evaluation and exploitation of the technologies and industrial property rights developed at the MDC.

Inventions and Existing Patent Portfolio of the MDC

Inventions from MDC employees are evaluated by Ascenion GmbH with regards to patents and all issues relevant to the market. In the years 2002 and 2003, patents were applied for in respect to 21 inventions from the MDC. The MDC patent portfolio comprises approximately 85 patent families. It consists of industrial property rights, quality to the extent of protection to be achieved, reachable markets, and the prospects for exploitation. The portfolio is characterized by high innovation earnings that are available to the MDC now.

Commercial Exploitation of Technologies

The commercialization of scientific results has taken on an increasing importance for the MDC and its employees in recent years. Thus, it was possible to increase the income from license and option contracts by more than 250,000 €. Additional income of over 190,000 € has been generated during the report period from agreements on transfer of tangible properties in respect to animal models.

Contact: Karen Uhlmann, Ascenion GmbH



„L'Homme“, Bronze, 1963, Jean Ipoustéguy
Copyright: MDC, Photographer: Gunter Lepkowski

Technologietransfer

In den Jahren 2002 und 2003 hat das MDC den Bereich Technologietransfer einer umfassenden Neustrukturierung unterzogen. Mit der Be- und Verwertung der am MDC entwickelten Technologien und Schutzrechte wurde ein externer Partner, die Ascenion GmbH, beauftragt.

Erfindungen und bestehendes Patentportfolio des MDC

Erfindungen von MDC-Mitarbeitern werden von der Ascenion GmbH hinsichtlich patent- und vor allem marktrelevanter Gesichtspunkte bewertet. In den Jahren 2002 und 2003 wurden 21 Erfindungen vom MDC zum Patent angemeldet. Das MDC-Patentportfolio umfasst etwa 85 Patentfamilien. Es besteht aus Schutzrechten, die einer eingehenden Begutachtung unter anderem hinsichtlich ihres Patentierungsverlaufs, der Qualität des zu erlangenden Schutzzumfanges, des erreichbaren Marktes und der Verwertungsaussichten unterzogen wurden.

Kommerzielle Verwertung von Technologien

Die Kommerzialisierung wissenschaftlicher Ergebnisse hat für das MDC und seine Mitarbeiter in den letzten Jahren einen immer wichtigeren Stellenwert eingenommen. So konnten die Einnahmen aus Lizenz- und Optionsverträgen um mehr als 250.000 € gesteigert werden. Aus Überlassungsverträgen für Tiermodelle wurden in diesem Zeitraum zusätzlich Einnahmen in Höhe von über 190.000 € generiert.

Kontakt: Karen Uhlmann, Ascenion GmbH

Press and Public Relations

The attention of the media and the public has been increasingly directed on biomedicine and gene research and, thus, ever increasingly on the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch. Primarily supported by federal taxpayer contributions, the MDC strives to communicate its work and progress to a broad public. To facilitate this communication, the MDC Press Office initiates the following activities.

The MDC Press Office published 124 press releases in German and English in 2002/2003. It was also responsible for the promotion of several international congresses, such as one for the 2002 recipient of the Nobel Prize for Chemistry, Prof. Kurt Wüthrich (Swiss Federal Institute of Technology (ETH) Zurich, Switzerland). Within the framework of the European Initiative for Communicators in Science (EICOS) for 2002 and 2003, the MDC Press Office organized and hosted the respective one-week visits of four journalists. In addition, in September 2003, the MDC Press Office prepared the visit of a group of ten Chinese journalists who were visiting Germany at the invitation of the German Federal Government.

During 2002/2003, the MDC Press Office generated over 6,000 newspaper articles, which corresponds to a circulation of more than 600 million. The topics conveyed to the media were broadcast by over 20 television stations in both regional as well as national transmissions. This included contributions to the German foreign broadcaster Deutsche Welle, to the Franco-German channel ARTE, to American Public Television, to the BBC (Europe Direct), as well as to the ZDF [Second German Channel], which, in its three-part series on "Gene Hunters", also reported on the German-Canadian research project of Prof. Ludwig Thierfelder on the cardiovascular research of the MDC/Franz-Volhard Clinic. In addition, over 30 radio contributions about the MDC were broadcast by public radio stations. Furthermore, online news services acquired a significant amount of information from the MDC Press Office via the Internet. The MDC Press Office arranged around 500 interviews for daily newspapers,

Presse- und Öffentlichkeitsarbeit

Das Augenmerk der Medien und der Öffentlichkeit richtet sich zunehmend auf die Biomedizin und damit immer stärker auch auf das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch. Das MDC wird mit öffentlichen Geldern finanziert. Es ist deshalb bemüht, der Öffentlichkeit und damit dem Steuerzahler in verständlicher Form zu vermitteln, für welche Forschungen es diese Mittel einsetzt.

124 Pressemitteilungen in Deutsch und Englisch recherchierte und verfasste die MDC-Pressestelle 2002/2003 und zeichnete auch verantwortlich für die Pressearbeit für mehrere internationale Kongresse. Dazu gehören auch Pressekonferenzen, die die MDC-Pressestelle inhaltlich und organisatorisch leitete, u. a. 2002 mit dem frisch gekürten Chemie-nobelpreisträger Prof. Kurt Wüthrich (Eidgenössische Technische Hochschule, ETH, Zürich/Schweiz). Im Rahmen der European Initiative for Communicators in Science (EICOS) bereitete die MDC-Pressestelle für 2002/2003 inhaltlich und organisatorisch die jeweils einwöchigen Besuche von vier Journalisten aus dem In- und Ausland vor und betreute sie vor Ort. Weiter bereitete sie im September 2003 den Besuch einer Gruppe von zehn chinesischen Journalisten vor, die auf Einladung der Bundesregierung eine Informationsreise durch Deutschland machten, und betreute sie zusammen mit MDC-Wissenschaftlern.

Die MDC-Pressearbeit war 2002/2003 Anlass für über 6.000 Zeitungsartikel, die einer Auflage von mehr als 600 Millionen entsprechen, in denen über die Forschungsarbeiten von MDC, und gemeinsamen Projekten von MDC, Kliniken und Biotechfirmen berichtet wurde. MDC-Themen wurden in über 20 Fernsehbeiträgen regionaler und überregionaler Sender aufgegriffen, darunter von der Deutschen Welle, dem deutsch-französischen Kanal ARTE, dem amerikanischen Public Television, der BBC (Europe Direct) sowie des ZDF, das in seiner dreiteiligen Serie über „Genjäger“ auch über ein deutsch-kanadisches Forschungsprojekt von Prof. Ludwig Thierfelder (MDC/Franz-Volhard-Klinik) berichtete. Weiter wurden über 30 Hörfunkbeiträge über das MDC gesendet. In großem Umfang griffen auch Online-Dienste im Internet



Aufmerksam verfolgen die beiden Kinder ein Experiment im „Chemietruck Justus“ des Bundesforschungsministeriums, der im Rahmen der Langen Nacht der Wissenschaften am 14. Juni 2003 auf den Campus Berlin-Buch gekommen war.

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Very concentrated watching an experiment on the “Chemistry truck Justus” of the Federal Research Ministry, which had come to the Berlin-Buch Campus on June 14, 2003.

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magazines, weekly newspapers, television, and radio as well as for scientific journals, such as *Nature* and *Science*. The Press Office also published eight press reports summarizing the coverage of the MDC and its collaborators. The MDC Press Office wrote several contributions for various brochures, including those for the Helmholtz Association, the Campus News of the BBB Management GmbH Berlin-Buch Campus, Partner for Berlin, as well as contributions to other magazines and book projects. In addition, the MDC Press Office updated the pages on the Internet in German and English which were adapted to reflect the new structure of the program-oriented funding of the Helmholtz Association (HGF).

Since January 2003, the Press Office has published a bilingual, internal Newsletter “News in Brief” (“Kurz und Knapp”) at irregular intervals to inform the employees about the most important developments at the MDC.

During the 2002/2003 period, the MDC Press Office organized a series of popular scientific Sunday lectures in the Berlin-Pankow Town Hall, started by the MDC in 1992. In addition, the MDC, along with the Berlin-Buch Campus, participated in Berlin’s citywide “Long Night of the Sciences”. Furthermore, the MDC Press Office hosted around 500 visitors, including students.

Head: Barbara Bachtler

Informationen der MDC-Pressestelle auf. Im Berichtszeitraum vermittelte die MDC-Pressestelle rund 500 Interviews für Tageszeitungen, Magazine, Wochenzeitungen, Fernsehen, Hörfunk sowie für wissenschaftliche Magazine wie *Nature* und *Science*, die sie inhaltlich und organisatorisch vorbereitete. Darüber hinaus gab die Pressestelle in dem genannten Zeitraum acht Pressespiegel heraus. Weiter verfasste die MDC-Pressestelle Beiträge für verschiedene Broschüren, darunter für die Helmholtz-Gemeinschaft, die Campus News der BBB Management GmbH Campus Berlin-Buch, Partner für Berlin, sowie für Buchprojekte. Zudem aktualisierte die MDC-Pressestelle im Berichtszeitraum die von ihr zu verantwortenden Seiten des Internets in Deutsch und Englisch, die an die neue Struktur der programmorientierten Förderung der Helmholtz-Gemeinschaft (HGF) angepasst wurden.

Seit Januar 2003 gibt die Pressestelle darüber hinaus in unregelmäßigen Abständen einen internen Newsletter „Kurz und Knapp“, „News in Brief“ heraus. Er informiert die Mitarbeiterinnen und Mitarbeiter in Deutsch und Englisch über die wichtigsten Entwicklungen im MDC.

Im Rahmen der Öffentlichkeitsarbeit führte die MDC-Pressestelle auch 2002/2003 die Serie populärwissenschaftlicher Sonntagsvorlesungen im Rathaus Berlin-Pankow fort, die das MDC 1992, dem Jahr seiner Gründung, initiiert hatte. Darüber hinaus beteiligte sich das MDC mit dem Campus Berlin-Buch im Berichtszeitraum an der in ganz Berlin durchgeführten „Langen Nacht der Wissenschaften“. Weiter betreute die MDC-Pressestelle 2002/2003 rund 500 Besucher, darunter Studenten und Schüler.

Leiterin: Barbara Bachtler

Administration

Verwaltung

Personnel

The MDC Personnel Department is responsible for all matters relating to staff, wages, salaries, separation allowances, staff hiring and removal, and travel expenses. Thus, the main duty of our department is to support to all employees and guests via counselling and educational training. We pay special attention to our foreign employees.

The MDC is currently financing 44 graduate students studying for a PhD. In addition, there are 69 part-time, third-party financed junior scientists. Among the 337 total scientists, 74 are foreigners representing 25 countries. As in the past, most (85 percent) of the scientists' contracts are limited to a maximum of five years.

Additional statistics regarding the personnel status can be found in the following diagram.

Head: Dr. Hans-Joachim Seehrich

Personal

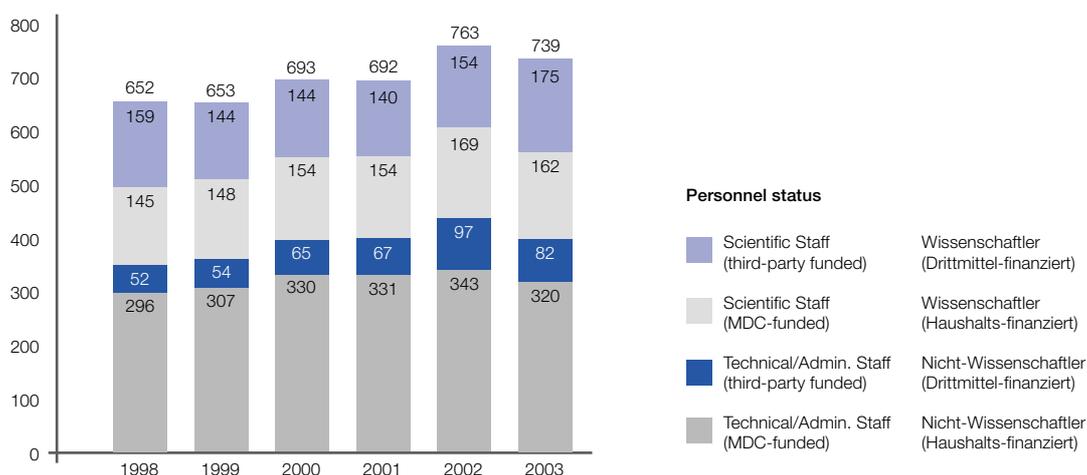
Die Mitarbeiterinnen und Mitarbeiter dieser Abteilung sind verantwortlich für den Abschluss von Arbeitsverträgen und alle damit im Zusammenhang stehenden Angelegenheiten. Hauptanliegen der Personalabteilung ist die Betreuung aller Mitarbeiter und Gäste des MDC durch Beratung und Fortbildungsangebote. Besonderes Augenmerk legen wir dabei auf unsere ausländischen Mitarbeiter.

Gegenwärtig sind am MDC 44 Doktoranden angestellt. Darüber hinaus werden aus Drittmitteln 69 teilzeitbeschäftigte Nachwuchswissenschaftler finanziert. Unter den 337 Wissenschaftlern kommen 74 aus 25 verschiedenen Ländern. Nach wie vor ist der Anteil der befristeten Verträge bei Wissenschaftlern mit 85 Prozent sehr hoch.

Die Angaben zum Personal der letzten Jahre und deren Finanzierung sind im folgenden Diagramm enthalten.

Leiter: Dr. Hans-Joachim Seehrich

Personnel status. Distinctions according to financial sources
Personal, unterschieden nach Finanzierung



Finances

The finance department concerns itself with all matters relating to the MDC's financial funding, including accounting. The primary source (90%) of the MDC's annual funding comes from the Federal government (Federal Ministry of Education and Research, BMBF) and the remaining 10% is provided by the State of Berlin (Senate Administration for Science, Research and Culture). Within the framework of its basic funding, the MDC received over 52 million Euros for the years 2002 and 2003. In addition, the MDC was able to increase its procurement of funds through third-party (external) financial sources from 13.2 million Euros in 2002 to 13.7 million Euros in 2003.

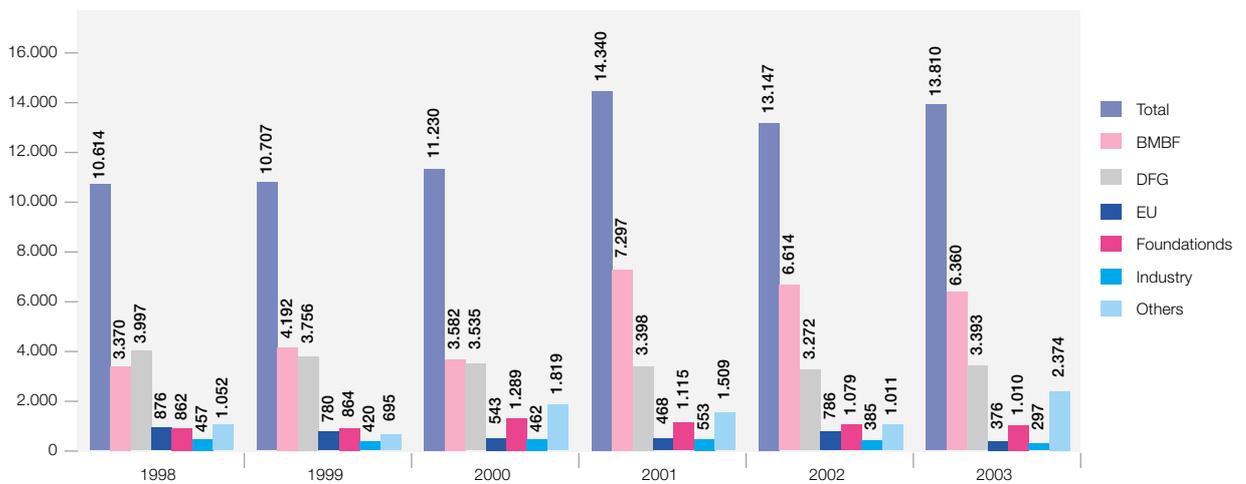
Head: Wolfgang Kühlewind

Finanzen

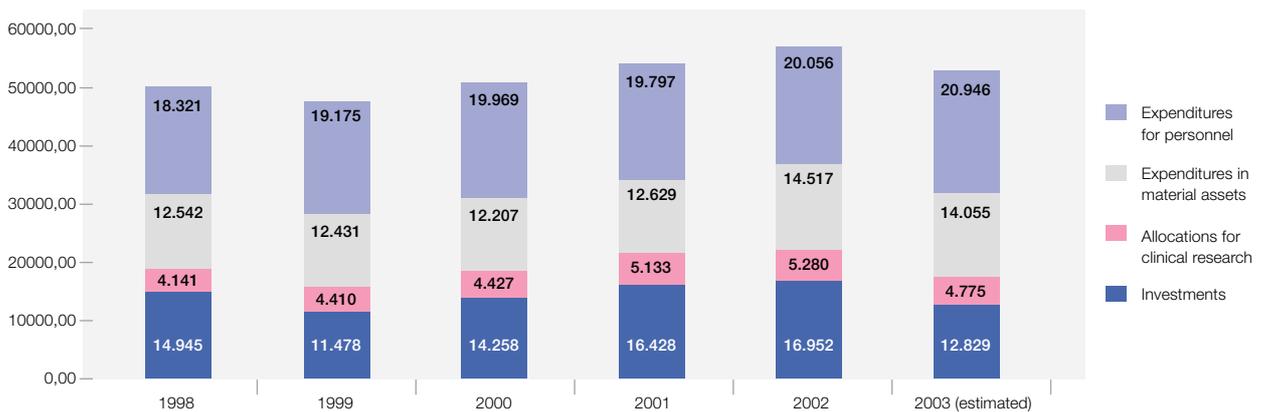
Die Finanzabteilung befasst sich mit allen Angelegenheiten der Einnahmen- und Ausgabenplanung des MDC, einschließlich des Rechnungswesens. Es wurden die Voraussetzungen geschaffen, dass die Kosten-Leistungs-Rechnung ab 2003 genutzt werden kann, um eine transparente Kostendarstellung und -verursachung anzuwenden. Der Hauptanteil (90 %) der jährlich verfügbaren finanziellen Mittel des MDC stammt aus dem Bundeshaushalt (Bundesministerium für Bildung und Forschung). Die verbleibenden 10 Prozent werden vom Land Berlin (Senatsverwaltung für Wissenschaft, Forschung und Kultur) geleistet. Im Berichtszeitraum 2002/2003 verfügte das MDC über eine Grundfinanzierung von über 52 Millionen Euro. Die positive Entwicklung bei der Einwerbung von Drittmitteln konnte fortgeführt werden. So war das MDC in der Lage, die Drittmittel von 13,2 Millionen Euro im Jahre 2002 auf 13,7 Millionen Euro im Jahre 2003 zu steigern.

Leiter: Wolfgang Kühlewind

MDC's Extra Mural Funding (in thousands of €)
Drittmittel des MDC (in tausend €)



Budget of the MDC (in thousands of €)
Budget des MDC (in tausend €)





Säule Durchgang Torhaus
 Copyright: MDC/Photograph: Gunter Lepkowski
 Column of the Gatehouse
 Copyright: MDC/Photographer: Gunter Lepkowski

Purchasing and Materials Management

The work of the Purchasing and Materials Management Department is focused on three main areas:

- Rapid and efficient supply of quality laboratory materials and equipment at cost-effective rates
- Step-by-step introduction of an electronic ordering department, enabling an effective and transparent purchasing process
- Revision and compilation of new, up-to-date rules of procurement

The step-by-step introduction of an electronic ordering system will ease the workload of the department. After hooking up to the company's internal data processing network, employees will be able to log in and place their orders themselves. This will guarantee the fast processing of purchasing orders. Linking the scientists' work places to the purchasing department is also an important requirement for the future transfer of data to contracted laboratory and specialist suppliers.

Head: Dr. Peter Konzer

Einkauf und Materialwirtschaft

Die Arbeit der Abteilung Einkauf und Materialwirtschaft des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch konzentrierte sich in den Jahren 2002 und 2003 auf drei Schwerpunkte:

- Rasche und bestmögliche Versorgung mit qualitätsgerechten Labor-, Hilfs- und Verbrauchsstoffen sowie Geräten unter Beachtung größtmöglicher Wirtschaftlichkeit
- Schrittweise Einführung eines elektronischen Bestellsystems zur effektiven und transparenten Gestaltung der Einkaufsvorgänge
- Überarbeitung und Neufassung der Beschaffungsordnung

Die schrittweise Einführung eines elektronischen Bestellsystems wird den Arbeitsaufwand der Mitarbeiter in der Abteilung entlasten. Nach Anschluss an das hausinterne Datenverarbeitungsnetz können die Mitarbeiter ihre Bestellanforderung selbst eingeben. Damit ist im Einkauf eine schnellere Bearbeitung gewährleistet. Die Vernetzung der Arbeitsplätze der Wissenschaftler mit dem Einkauf ist auch eine wichtige Voraussetzung für die zukünftige Datenfernübertragung mit entsprechenden Labor- und Fachhändlern nach Abschluss von Rahmen- und Abrufverträgen.

Leiter: Dr. Peter Konzer

Central Facilities

Library

The MDC library is a specialized scientific library involved in providing and supplying information in all research areas of the MDC and its associated clinics. The library acts as a modern information centre using advanced network structures and allowing access to information sources around the world.

Modern information networks offer increased opportunities to supply users with specific literature and information resources at their work place. The collection contains more than 45,700 items and 230 print periodicals as well as different kinds of non-print materials. In addition to the print subscriptions, the library offers more than 1,000 electronic journals. Campus-wide provision of major local databases include Medline (starting from 1966) and Web of Knowledge (including 5 editions of Current Contents Connect and Web of Science starting from 1980) and CD-Rom databases via internal MDC-network with a range of scientific options.

The open area provides 26 reading desks, 5 computer workstations with Internet access, and 3 access points for laptops. The library operates with the local library computer system SISIS. The OPAC (Online public access catalogue) lists all collections and is also available via the Internet. Printed catalogues describing older holdings with alphabetical and classified indices are also available while the library and its services are all available via the Internet. The client-server architecture provides fast access, regardless of the user's operating system. The library offers a selection of main links related to research on the campus.

Head: Dr. Dorothea Busjahn

Zentrale Dienste

Bibliothek

Die Bibliothek des Max-Delbrück-Centrums für Molekulare Medizin (MDC) Berlin-Buch ist eine moderne wissenschaftliche Spezialbibliothek, die die Forschung am MDC mit der Bereitstellung von Literatur, Online-Informationsvermittlung aus externen Datenbanken sowie der Lieferung von Informationen in gedruckter und anderer Form unterstützt.

Sie arbeitet als eine Mischform aus Präsenzbibliothek mit Freihandaufstellung und Ausleihe mit offenem Magazin. Der Gesamtbestand beträgt über 45 700 Medieneinheiten und 230 laufend gehaltene Periodica, zusätzlich über 1000 Titel in elektronischer Form. Unter den wichtigsten Literaturdatenbanken sind Medline (Zugriff ab 1966, Web of Knowledge incl. Current Contents Connect (in 5 Ausgaben), Web of Science (Zugriff ab 1980) und der Science Citation Index.

Der Freihandbereich verfügt über 26 Arbeitsplätze, 5 Computer-Plätze mit Internet-Zugang und 3 Plätze, die einen laptop-Anschluss gestatten. Als lokales Bibliothekssystem arbeitet das Datenverwaltungs- und Informationssystem SISIS. Der OPAC (Online Public Access Catalogue) verzeichnet den gesamten Bibliotheksbestand und ist über das Internet recherchierbar. Durch die Einbindung des Bibliotheksnetzes in das Campusnetz stehen alle elektronischen Informationsangebote campusweit zur Verfügung. Durch seine Client-Server-Architektur gestattet das Bibliotheksnetz seinen Nutzern einen schnellen, oberflächenunabhängigen und direkten Zugriff zu den aufliegenden Datenbanken. Über ihre Homepage präsentiert sich die Bibliothek im Internet, stellt dort eine Auswahl ihrer Dienste zur Verfügung und bietet eine Reihe dem Forschungsprofil angepaßter links.

Leiterin: Dr. Dorothea Busjahn



„Großes Sonnenzeichen I“, Bronze, 1995, Rainer Kriester, vor dem MDC.C
 Copyright: MDC, Photograph: Gunter Lepkowski
 „Large Sun Drawing I“, Bronze, 1995, Rainer Kriester, in front of the MDC.C
 Copyright: MDC, Photographer: Gunter Lepkowski

Animal Facilities

Among the research institutes in Berlin, the MDC animal facility possesses the most genetically engineered rat and mice stocks. The four animal houses support transgenic and animal experimentation at the MDC. Mice and rats are bred in a disease-free environment. More than 400 strains of knock-out mice are now available as experimental models in cancer, cardiovascular, and neurological research. Encompassing an area of 1,260 square-meters, the facilities include animal rooms (636 m²), surgery rooms, storage rooms, and cage washing facilities. Nevertheless, the number of available animal rooms and surgical facilities in and around the MDC does not meet present demands.

The rapidly growing number of genetically engineered models of severe human illness means that further animal facilities for breeding and experimentation are needed. Therefore, the MDC is building a new central animal house. This new facility will possess substantially more capacity for animals (29 animal rooms covering a 1,015 m² area) and laboratories (19 labs over 665 m²) and will be opened during the year 2004.

Head: Dr. Karin Jacobi

Tierhaltung

Unter den Forschungsinstituten Berlins besitzt die MDC-Versuchstierhaltung die meisten gentechnisch veränderten Rattenlinien und Mäusestämme. Mehr als 400 Knock-out-Stämme sind gegenwärtig verfügbar als experimentelle Modelle für die Erforschung von Krebs-, Herz-Kreislauf- und neurologischen Erkrankungen. Die 5 Tierhäuser des MDC versorgen die transgenen Versuchstiere und die dazugehörigen Experimente. Mäuse und Ratten werden unter SPF-Bedingungen gezüchtet und gehalten. Lokalisiert auf 1.260 Quadratmeter Fläche, enthalten die Tierhäuser Tierräume (636 Quadratmeter), Labore, Lagerräume und Räume für die Käfigreinigung. Trotz dieser umfangreichen Flächen kann der Bedarf für die Wissenschaftler nicht erfüllt werden.

Die schnell wachsende Zahl genetisch modifizierter Modelle für verschiedene Humanerkrankungen bedeutet, dass weitere Tierhaltungsflächen zur Zucht und Haltung im Experiment erforderlich sind. Deshalb hat das MDC ein neues zentrales Tierhaus gebaut, das im Laufe des Jahres 2004 fertiggestellt und bezogen wird. Die neue Tierhaltung wird substantiell mehr Kapazität für Tiere (29 Tierräume mit einer Fläche von 1.015 Quadratmetern) und Labore (19 Labore mit 665 Quadratmetern Fläche) besitzen.

Leiterin: Dr. Karin Jacobi

Data and Image Processing

The MDC Department of Data and Image Processing is responsible for the information technology (IT) deployment in the administration and supports the central scientific infrastructure. Thus, the Group takes care of the Central Server of the MDC and makes possible the use of electronic files, digital backups, remote access, and web services. The Group is also responsible for the Client-/Server operation of the administration including the SAP System and user support. The completed migration and expansion of the SAP system in 2003 was linked to the introduction of the cost and performance accounting and electronic purchasing systems. In the future, we intend to strengthen the deployment of server-based technologies such as Citrix-Metaframe. Currently, the Group is working on the introduction of indexing services and the improvement of the electronic and data security within the MDC Computer Network.

Head: Bernd Lemke

Maintenance and General Services

The Department is in charge of the Appliance Service Groups as well as all central glassware-cleaning rooms of the MDC. In the period of the report, the operation of the telephone system was converted to WINDOWS 2000-oriented software, enabling a very flexible operation independent of location. A further achievement was the revision of the interlinked fire alarm systems in the MDC buildings. Recently, documentation of the MDC buildings has been drawn up and all areas recorded according to *DIN* [German Industrial Standard] 227 jointly with the BBB Management GmbH Campus Berlin-Buch under the vision of modern "Facility Management".

Head: Harry Schenk

EDV und Bildverarbeitung

Im MDC ist die Gruppe EDV und Bildverarbeitung verantwortlich für den IT-Einsatz in der Verwaltung und unterstützt die zentrale wissenschaftliche Infrastruktur. So betreut die Gruppe die zentralen Server des MDC und ermöglicht den Forschungsgruppen die Nutzung von File-, Backup-, RemoteAccess- und Web-Diensten. Ebenso ist die Gruppe verantwortlich für den Client-/Server-Betrieb der Verwaltung einschließlich der SAP-System- und -Nutzerbetreuung. 2003 wurde die Migration und Erweiterung des SAP-Systems abgeschlossen, welche mit der Einführung der Kosten- und Leistungsrechnung und des Elektronischen Einkaufs verbunden ist. Künftig wollen wir den Einsatz Serverbasierter Technologien wie Citrix-Metaframe verstärken. Gegenwärtig ist die Gruppe mit der Einführung von Verzeichnisdiensten und der Verbesserung der Sicherheitslösung im MDC-Computer-Netzwerk beschäftigt.

Leiter: Bernd Lemke

Haustechnik

Der Abteilung Haustechnik sind die Gruppe Geräteservice sowie sämtliche zentralen Spülküchen des MDC Berlin-Buch unterstellt. Im Berichtszeitraum wurde die Bedienung der Telefonanlage auf eine WINDOWS 2000-orientierte Software umgestellt. Damit ist eine sehr flexible, vom Standort unabhängige Bedienung, möglich. Ein weiterer Schwerpunkt war die Überarbeitung der vernetzten Brandmeldeanlagen in den MDC-Gebäuden. In der zurückliegenden Zeit wurde gemeinsam mit der BBB Management GmbH Campus Berlin-Buch unter dem Gesichtspunkt eines modernen „Facility Management“ eine Dokumentation der MDC-Gebäude erarbeitet und alle Flächen nach DIN 227 erfasst.

Leiter: Harry Schenk

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