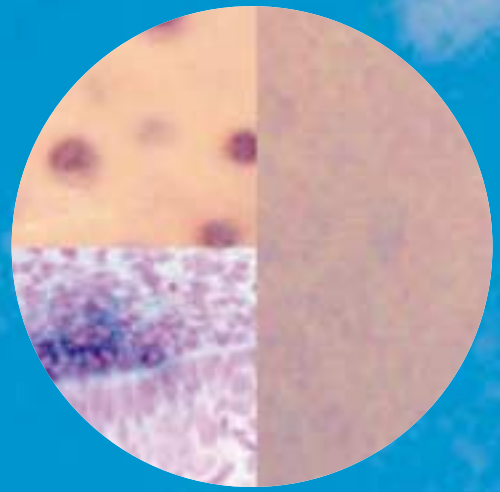

Research Report 2002

MDC
Berlin-Buch

Max Delbrück Center for Molecular Medicine

Research Report 2002
(covers the period 2000-2001)

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Molecular Medicine (MDC)
Berlin-Buch

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Introduction

Einführung

Molecular Medicine – A Concept and its Application at the MDC in Berlin-Buch

History and Foundation

On January 1, 2002, the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch celebrated its 10th birthday. It was founded on January 1, 1992, from the Institutes of the East German Academy of Sciences, the Central Institute for Cancer Research, the Central Institute for Cardiovascular Research and the Central Institute for Molecular Biology. The center is named after Max Delbrück, a native of Berlin, and one of the founding fathers of molecular biology who won the Nobel Prize in 1969. Delbrück collaborated at the Kaiser Wilhelm Institute for Brain Research in Berlin-Buch with the Russian geneticist, N. W. Timoféev-Ressovsky and the physicist, K.G. Zimmer. In 1935, their interdisciplinary research led to the publication of the epoch-making paper "The Nature of Gene Structure and Gene Mutation".

Anyone who experienced the reunification of Germany between November 9, 1989, and January 1, 1992 – involving the break up of the GDR, the unification of the two Germanies, with the evaluation of the entire scientific system of the GDR, the many plans for the restructuring of the scientific establishments and institutional and personal uncertainties of every kind – can imagine the problems people experienced, as well as the hope and expectations during this two-year period. In 1991, in Berlin-Buch, there were over 1,000 staff, physicians, researchers, and technicians working in the institutes of the Academy and the clinics. At its foundation, the MDC had 350 budgeted posts. In such circumstances it was no simple matter to set up a new organization to match the standard of the former institutes of the Academy and guarantee all qualified staff a future post. The details of this critical and exciting period of the restructuring of science on the Buch Campus has been comprehensively described else-

Molekulare Medizin – Ein Konzept und seine Umsetzung am MDC in Berlin-Buch

Geschichte und Gründung

Am 01. Januar 2002 beging das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch sein 10-jähriges Bestehen. Es ist am 01. Januar 1992 aus den Instituten der Akademie der Wissenschaften der DDR, dem Zentralinstitut für Krebsforschung, dem Zentralinstitut für Herz-Kreislaufforschung und dem Zentralinstitut für Molekularbiologie hervorgegangen. Die Benennung des Institutes erfolgte nach Max Delbrück, dem aus Berlin stammenden Wegbereiter der Molekularbiologie, der 1969 mit dem Nobelpreis ausgezeichnet worden war. Delbrück hatte am Kaiser-Wilhelm-Institut für Hirnforschung in Berlin-Buch zusammen mit dem russischen Genetiker N. W. Timoféeff-Ressovsky und dem Physiker K. G. Zimmer gearbeitet, und bei dieser interdisziplinären Forschung war 1935 die epochemachende Arbeit über die „Natur der Genstruktur und der Genmutation" entstanden.

Wer die Zeit der deutschen Wiedervereinigung zwischen dem 09. November 1989 und dem 1. Januar 1992 persönlich miterlebt hat – mit dem Zusammenbruch der DDR, mit dem Prozess der Vereinigung der beiden deutschen Staaten, mit der Evaluation des gesamten Wissenschaftssystems der DDR, mit den vielen Plänen für die Neustrukturierung der wissenschaftlichen Einrichtungen und mit den Unsicherheiten in institutioneller und persönlicher Art –, kann sich vorstellen, welche Belastungen, aber auch welche Hoffnungen und Erwartungen in diesen zwei Jahren des Prozesses der deutschen Wiedervereinigung durchlebt wurden. In Berlin-Buch waren 1991 weit über 1.000 Mitarbeiter, Ärzte, Wissenschaftler, Technische Angestellte in den Akademieinstituten und Kliniken beschäftigt. Das MDC hatte bei der Gründung 350 budgetierte Stellen. Es war in dieser Situation nicht einfach, eine dem Leistungsniveau der Akademieinstitute entsprechende neue

where. It was only possible because everyone was sympathetic to the final aim and worked together to ensure that it was achieved.
The significant and unavoidable problems at personal and institutional levels were made easier by the fact that everyone was aware of the great tradition of the Buch Institutes which had supported outstanding researchers like Oskar and Cécile Vogt, Walter Friedrich, Karl Lohmann, Arnold Graffi, Erwin Negelein, Albert Wollenberger and Hans Gummel. The real and positive role played by tradition in building new structures at a time of great change can be particularly well observed and experienced in Berlin. Attention to tradition was and still is important as far as setting up the MDC and the Berlin-Buch Campus are concerned.

The clinics of the Academy of Sciences were incorporated into the university system – thanks to the energetic efforts of the then Senator for Science, Prof. Manfred Erhardt – as the Robert Rössle Cancer Clinic and the Franz Volhard Cardiovascular Clinic of the Charité, Berlin-Buch Campus. Thanks to particularly well defined cooperation contracts, they were linked to the MDC in both personnel and institutional terms. BBB Biotechnologie Berlin-Buch Management GmbH set up a biotechnology park. This has allowed the basic research activities at the MDC, and clinical research at the Charité clinics and the Buch Clinic as well as the Technology Park established by BBB Management GmbH to develop a synergy with respect to all research efforts that has subsequently been shown to be successful based on any number of criteria. The pure research carried out on the Berlin-Buch Campus was boosted in1999 by the arrival of the Forschungsinstitut für Molekulare Pharmakologie (FMP; Research Institute for Molecular Pharmacology), which is part of the Wilhelm Gottfried Leibniz Association. The institute collaborates closely with the MDC on structural research into biologically active molecules. The privatization of the clinics under the auspices of Helios Kliniken GmbH in summer 2001 has created even more opportunities for cooperation.

Bundespräsident Johannes Rau (Mitte) im Gespräch mit Prof. Detlev Ganten (MDC-Stiftungsvorstand) und dem damals designierten und jetzigen Administrativen Vorstand des MDC, Dr. Waltraud Kreutz-Gers, anläßlich der Verleihung des Deutschen Zukunftspreises des Bundespräsidenten am 29. November 2001 im MDC Kommunikationszentrum.
The President of the Federal Republic of Germany, Johannes Rau (in the middle), chats with the Scientific Director of the MDC, Detlev Ganten (MD. Ph.D), and the then Administrative Director designate, Dr. Waltraud Kreutz-Gers, after the awarding of the President's "Prize for Technology and Innovation" which took place at the MDC's new Max Delbrück Communications Center (MDC.C) on November 29, 2001.

Organisationsform zu finden und allen qualifizierten Mitarbeitern die ihnen zukommende Position zuzusichern. Über Einzelheiten dieser entscheidenden und aufregenden Monate und Jahre der Neuorientierung der Wissenschaft auf dem Campus Buch ist an anderer Stelle ausführlich berichtet und geschrieben worden. Sie konnten nur bewältigt werden, weil es von allen Seiten viel Verständnis und Unterstützung gab.
Die großen und wohl unvermeidbaren Brüche in institutioneller und personeller Hinsicht wurden unter anderem dadurch erleichtert, dass ganz bewusst die große Tradition der Bucher Institute mit so herausragenden Wissenschaftlern wie Oskar und Cécile Vogt, Walter Friedrich, Karl Lohmann, Arnold Graffi, Erwin Negelein, Albert Wollenberger und Hans Gummel fortgeführt wurde. Die reale und konkrete positive Bedeutung der Tradition für die Zukunftsgestaltung in einer Zeit großer Umbrüche, kann in Berlin besonders gut beobachtet und erlebt werden. Traditionspflege wurde und wird bewußt für den Aufbau des MDC und des Campus Berlin-Buch eingesetzt.

Die Kliniken der Akademie der Wissenschaften wurden mit tatkräftiger Unterstützung des damaligen Senators für Wissenschaft, Prof. Manfred Erhardt, in das universitäre System eingegliedert als Robert-Rössle-Krebsklinik und Franz-Volhard-Herz-Kreislaufklinik der Charité, Campus Berlin-Buch. Durch einen besonders engen Kooperationsvertrag wurden sie mit dem MDC personell und institutionell verbunden. Der Aufbau eines Biotechnologieparks wurde durch die BBB Biotechnologie Berlin-Buch Management GmbH begründet. Auf diese Weise wurde mit der Grundlagenforschung am MDC, der klinischen Forschung an den Charité-Kliniken und dem Klinikum Buch sowie mit dem von der BBB Management GmbH eingerichteten Technologiepark ein Synergien schaffendes komplettes Forschungssystem eingerichtet, das sich in den folgenden Jahren in jeder Hinsicht als erfolgreich erwiesen hat. 1999 wurde die Grundlagenforschung auf dem Campus Berlin-Buch durch den Zuzug des Forschungsinstituts für Molekulare Pharmakologie (FMP), das zur Wilhelm-Gottfried-Leibniz-Gemeinschaft gehört, verstärkt. Dieses Institut arbeitet mit dem MDC insbesondere auf dem Gebiet der Strukturforschung für biologisch aktive Moleküle intensiv zusammen. Die Privatisierung der Kliniken durch die Helios Kliniken GmbH im Sommer 2001 schafft erweiterte Möglichkeiten der Kooperation.

Aufbruchstimmung in Berlin-Buch

Auf dem Campus Berlin-Buch herrscht Aufbruchstimmung: Das Max Delbrück Communications Center (MDC.C) mit Hörsälen, Seminar- und Laborräumen für Kongresse, Fortbildung und Kursen ist im Jahre 2001 fertig gestellt und in Betrieb genommen worden. Der erste Bauabschnitt des Helmholtz Hauses für Tierlaboratorien und Büroräume wird noch im Jahre 2002 bezogen. Ein neues Zentrum für Medizinische Genomforschung des MDC ist im Entstehen. Vier neue Laborgebäude für Existenzgründer und Biotechnologiefirmen sind fertig gestellt bzw. befinden sich zur Zeit im Bau. Das neue „Helios Klinikum Berlin" wird auch die universitären Kliniken der Charité in einem Neubau für 400 Millionen Mark mit aufnehmen, so dass eine der modernsten und größten Kliniken Berlins in unmittelbarer Nachbarschaft des Forschungscampus

Developments in Berlin-Buch

Spirits on the Berlin-Buch Campus are high as the site develops: the Max Delbrück Communications Center (MDC.C), with its lecture theaters, seminar rooms and laboratory space for congresses, further education and training courses, was opened in 2001. The first section of the "Helmholtz Haus" for animal laboratory facilities and offices will be ready in 2002. A new MDC Center for Medical Genome Research is being created. Four new laboratory buildings for start-up companies and biotechnology companies have been completed or are under construction. The new "Helios Klinikum Berlin" will also incorporate the university clinics of the Charité in a new setting costing approximately 200 million Euro so that one of the most modern and largest clinics in Berlin will be located right next to the research campus. The space occupied by the clinics previously represents an area of over 100 ha and this will allow the Technology Park to expand. The clinic buildings designated as being of historic interest will be used as a "Cité Universitaire", and will be adopted by the European College of Liberal Art (ECLA). A new shopping center will be built right next to the S-Bahn station. It is also planned to build a hotel for visitors and congress delegates as well as relatives of the patients being treated in the clinics.

The health theme, which has made Berlin-Buch, the "Health Region" is becoming more and more important as a part of the modern knowledge-based society. In the future, Berlin-Buch will be a center for molecular medicine where the latest advances in genome research and gene technology will be applied to the clinical setting and in the field of business, allowing biotech companies and service firms to develop and create new jobs.

The Sculpture Park on the Berlin-Buch Campus with works by, among others, Ipousteguy, Szymanski and Kriester, reflects the close relationship between the Arts and Sciences, which is particularly encouraged in Berlin-Buch. In this way, Buch will be seen as an intellectual focal point where the development of molecular medicine and gene technology is being advanced alongside a continuing dialog with people from many different backgrounds. In this way Berlin-Buch will become a model of a future human knowledge-based society. On the MDC Campus over 2000 staff are currently working in the research institutes, clinics and biotech companies, many more than before reunification in 1989. During the last 10 years many new jobs have been created in this science-based environment.

The rapid connections linking Berlin-Buch to the cultural center of central Berlin and the proximity of the Barnim Nature Conservation Park have made Berlin-Buch one of the most attractive and dynamic suburbs of the new Berlin.

Many of the staff who work in the research institutes and the biotech companies live only 15 minutes away in Prenzlauer Berg, one of the favorite parts of Berlin with many theaters, bars and cinemas. Prenzlauer Berg is close to the cultural and spiritual heart of the city with the Humboldt University, the Museum Island, the main theaters, the Berlin Philharmonic and the Kulturforum.

entstehen wird. Die freiwerdenden Klinikgebäude in einem Areal von über 100 Hektar werden für den expandierenden Technologiepark zur Verfügung stehen. Denkmalgeschützte Klinikgebäude sollen für eine „Cité Universitaire", die auch das European College of Liberal Art (ECLA) aufnehmen wird, genutzt werden. In unmittelbarer Nähe des S-Bahnhofes wird ein neues Einkaufszentrum gebaut. Dazu ist geplant, ein Hotel zu errichten für Besucher und Kongressteilnehmer sowie für Angehörige von Patienten der Kliniken.

Das Thema Gesundheit, dem sich Berlin-Buch, die „Gesundheitsregion", verschrieben hat, wird als Teil der modernen Wissensgesellschaft immer weiter an Bedeutung gewinnen. Berlin-Buch soll in Zukunft ein Zentrum für die Molekulare Medizin sein, in dem die Fortschritte der Genomforschung und Gentechnik verantwortungsvoll in Klinik und Wirtschaft angewendet werden und um das herum sich Biotechnologiefirmen und Serviceeinrichtungen entwickeln und neue Arbeitsplätze entstehen lassen. Der Skulpturenpark auf dem Campus Berlin-Buch mit Werken unter anderem von Ipousteguy, Szymanski und Kriester ist Ausdruck der engen Ver-

Dr. Erwin Jost, Administrativer Vorstand des MDC, Dr. Arend Oetker (Vorsitzender des Kuratoriums Deutscher Zukunftspreis und Präsident des Stifterverbandes) und Prof. Detlev Ganten (MDC-Stiftungsvorstand) beim Empfang nach der Vergabe des Zukunftspreises des Bundespräsidenten am 29. November 2001 im neuen Max Delbrück Communications Center (MDC.C)
Reception after the awarding of the "Federal President's Prize for Technology and Innovation" in the new Max Delbrück Communications Center (MDC.C) on November 29, 2001 (from left): Dr. Erwin Jost (Administrative Director of the MDC), Dr. Arend Oetker (Chairman of the Board of Trustees of the President's "Prize for Technology and Innovation" and President of the Stifterverband für die Deutsche Wissenschaft – Donor's Association for the Promotion of the Sciences and Humanities) and Detlev Ganten (MD., Ph.D, MDC's Scientific Director).

Buch is a place of synergies. As a link between town and country, between pure research and the clinics as well as scientific applications of this research, living, working and relaxing in Berlin-Buch are all equally attractive.

The concept of molecular medicine

As far as the success of any scientific institution is concerned, the key factors are the original idea upon which it is based, the efforts of its scientists and other key staff, their commitment, the quality of the research activities, projects and its publication record.

Abschied nach acht Jahren MDC: Prof. Jutta Schnitzer, wissenschaftliche Referentin des MDC und jetzige Generalsekretärin der Leopoldina, Halle. MDC-Vorstand Prof. Detlev Ganten beim Überreichen des Blumenstrausses.
Farewell after eight years at the MDC: Prof. Jutta Schnitzer, MDC`s former Scientific Coordinator and now Secretary General of the "Leopoldina" (Halle) receives a bouquet of flowers from Detlev Ganten (MD., PhD), MDC`s Scientific Director
Copyright: MDC; Siegfried Endruweit

When the MDC was founded, we took advice based on the recommendations of the Scientific Council and the Founders Committee regarding ideas about the long-term developments in medicine and the latest results of molecular genetics and genome research.

There are two reasons for the extraordinary successful and sustained development of the modern life sciences. On the one hand, there were the scientists themselves, who highlighted the new, often physico-chemical, methods which could identify the macromolecules which had key biological functions in biology and medicine, thereby allowing a better understanding of the interplay between the form and function of cells. One the other hand, institutions like the Rockefeller Foundation play an important role. For example, in 1938, it supported a research program for the advancement of genetics giving it the name "molecular biology". One aim of this project was to raise the level of biosciences at that time and turn it into a quantitative "Science of Man", which went beyond simply describing events that happened in Nature. Over the period 1937–39, the person who gave his name to the MDC, Max Delbrück, held a fellowship from the Rockefeller Foundation and their support helped him become one of the

bindung von Kunst und Wissenschaft, die in Berlin-Buch besonders gepflegt wird. Buch soll sich auf diese Weise als intellektueller Kristallisationspunkt profilieren, in dem die Entwicklung der Molekularen Medizin und Gentechnologie vorangebracht und durch den Dialog mit breiten Kreisen der Gesellschaft kritisch begleitet wird. Auf diese Weise wird Berlin-Buch zu einem Modell für die zukünftige humane Wissensgesellschaft. Auf dem Campus Berlin-Buch arbeiten zur Zeit in den Forschungseinrichtungen, Kliniken und Biotechnologiefirmen über 2.000 Mitarbeiter, weit mehr als vor der Wiedervereinigung 1989. Im Umfeld der Wissenschaft sind damit in den vergangenen 10 Jahren viele neue anspruchsvolle und zukunftsfähige Arbeitsplätze geschaffen worden.

Die schnelle Anbindung von Berlin-Buch an das kulturelle Zentrum in Berlin-Mitte und die direkte Nachbarschaft zum Naturschutzpark Barnim machen Berlin-Buch zu einem der attraktivsten und dynamischsten Vororte des neuen Berlin.
Viele Mitarbeiter der Forschungseinrichtungen und Biotechnologiefirmen wohnen in dem 15 Minuten entfernten Prenzlauer Berg, einem der beliebtesten Stadtteile Berlins mit vielen Theatern, Kneipen und Kinos. Dem Prenzlauer Berg schließt sich direkt das kulturelle und geistige Zentrum der Stadt an mit der Humboldt Universität, der Museumsinsel, den großen Theatern, der Philharmonie und dem Kulturforum.

Buch ist ein Ort der Synergien. Als Nahtstelle zwischen Stadt und Land, zwischen Forschung und klinischer sowie wirtschaftlicher Anwendung ist das Leben, das Arbeiten und das Erholen in Berlin-Buch gleichsam attraktiv.

Das Konzept der Molekularen Medizin

Entscheidend für den Erfolg einer wissenschaftlichen Institution ist das inhaltliche Konzept, die wissenschaftliche Leistung, die tragenden Personen, das Engagement der Wissenschaftlerinnen, Wissenschaftler und Mitarbeiter, die Qualität der Forschungstätigkeit, Projekte und Publikationen.

Zur Zeit der Gründung des MDC haben wir uns auf der Basis der Empfehlungen des Wissenschaftsrates und des Gründungskomitees bei den Konzepten von langfristigen Entwicklungen der Medizin und neuesten Ergebnisse der molekularen Genetik und Genomforschung leiten lassen.

Es gibt zwei Quellen für das außerordentlich erfolgreiche und nachhaltige Konzept der modernen Lebenswissenschaft. Zum einen waren dies die Wissenschaftler selbst, die mit neuen, über die Biologie und Medizin hinausreichenden, häufig physikalisch-chemischen Methoden die Strukturen von Makromolekülen mit wichtigen biologischen Funktionen erkunden konnten und sich anschließend daran machten, das Wechselspiel von Form und Funktion in der Zelle zu verstehen. Zum zweiten spielten Institutionen wie etwa die Rockefeller Stiftung eine wichtige Rolle, die im Jahre 1938 einem Forschungsprogramm zur Förderung der Genetik den Namen „Molekularbiologie" gab. Ein Ziel dieser Initiative lag darin, das Niveau der damaligen Biowissenschaften anzuheben und zu einer quantitativ operierenden „Science of Man" auszuweiten, die über die reine Beschreibung der Natur hinausging. Der Namenspatron des MDC, Max Delbrück, gehörte in

founding fathers of molecular biology; there is much that we can still learn from him today.

When the MDC was established ten years ago in Berlin-Buch and raised a flag as far as the development of molecular medicine was concerned, there were only a few institutes around the world engaged in applying the latest results of pure research to medical research in a closely-knit institutional setting. At that time, no one could have known the kind of progress that would take place in genome analysis in the next ten years. The mission of the MDC is to combine modern medical and clinical research with the methods of molecular biology and cell biology as well as the expansion in gene technology, which systematically concentrates on the genome and the latest results arising from research into it. As far as pure research is concerned, key areas should be those that are of vital importance for the analysis of disease phenomena and which lead to new opportunities firmly based on the natural sciences for diagnosis, treatment and prevention.

den Jahren 1937–39 zu den Stipendiaten der Rockefeller Stiftung und ist mit ihrer Hilfe zu dem Wegbereiter der Molekularbiologie geworden, von dem wir heute noch lernen können.

Als das MDC vor zehn Jahren in Berlin-Buch gegründet wurde und sich die Entwicklung einer Molekularen Medizin auf die Fahnen schrieb, gab es weltweit nur wenige Institute, die so konsequent die Anwendung neuester Methoden in der Grundlagenforschung für medizinische Forschung in enger institutioneller Verbindung verfolgten. Niemand konnte damals wissen, welche Fortschritte die Genomanalyse in diesem Zeitraum machen würde. Die Aufgabe des MDC bestand darin, moderne medizinische und klinische Forschung im Verband mit molekularbiologischen, zellbiologischen sowie zunehmend gentechnischen Methoden zu betreiben, die sich systematisch auf das Genom konzentrierten und von ihm ausgingen. Wegweisend für die Grundlagenforschung sollten Themen sein, die von prinzipieller Bedeutung für die Analyse von Krankheitsphänomenen sind und aus denen sich naturwissenschaftlich begründet neue Möglichkeiten für Diagnostik, Therapie und Prävention ableiten lassen.

The multitalented molecules of molecular medicine

Touch and Pain: two senses, one ion channel named DRASIC

Our senses of touch and pain, opposites one might think, have something in common, an ion channel called DRASIC. DRASIC, which stands for dorsal root ganglion acid sensitive channel, has been shown to be critical for both these senses. The DRASIC protein forms a channel in the membrane that when opened conducts sodium ions which then leads to the electrical excitation of neurons. A first clue about the possible function of the DRASIC protein came from a genetic screen for mutations in the nematode worm C.elegans which showed that similar proteins in the worm are necessary for the worm to sense touch. These proteins including DRASIC are all members of the Deg/Enac channel superfamily. Thus arose the idea that mechanical forces on the membrane could open the channel: this would make it a mechanical sensor. Thus the same channel obviously helps sensory neuron to detect innocuous and painful stimuli in a completely different way. It is known that DRASIC can potentially form heteromeric ion channel complexes with other members of the Deg/Enac family of proteins.

Another aspect of DRASIC channel function is the fact that acidification ie. protons are a very effective stimulus to open the channel. Acidification is a universal consequence of injury or inflammation of peripheral tissues. Mice lacking DRASIC were much less affected by muscle acidification, in other words the lack of the channel protein had an analgesic effect. The diversity of function for the DRASIC channel reported here for the first time shows directly that the channel does not work alone but in concert with other as yet unknown proteins. It is precisely this kind of in vivo analysis of protein function that highlights the principle that the function of a single protein can vary enormously depending on the cellular context. Thus products of single genes are not simple entities that work alone to accomplish a well-defined function but rather execute their function in a multitude of ways depending the other proteins that they can interact with. For further details see *Gary Lewin's* research group.

Die vielseitigen Moleküle der Molekularen Medizin

Berührung und Schmerz: Zwei Sinne, ein Ionenkanal namens DRASIC

Der Tastsinn und der Schmerz erscheinen zwar als gegenteilige Sinne, sie haben aber etwas gemeinsam, einen Ionenkanal mit Namen DRASIC. DRASIC kürzt den englischen Ausdruck „dorsal root ganglion acid sensitive channel" ab, womit ein säureempfindlicher Ionenkanal im dorsalen Wurzelganglion gemeint ist. Es konnte gezeigt werden, dass DRASIC für die beiden genannten Sinne wesentlich ist. Das DRASIC Protein formt einen Kanal durch die Membran, durch den im geöffneten Zustand Natriumionen strömen, die dann zur elektrischen Erregung von Neuronen führen. Einen ersten Hinweis auf die mögliche Funktion des DRASIC Proteins ergab eine genetische Reihenuntersuchung von Mutationen in dem Fadenwurm C. elegans. Sie zeigte, dass ähnliche Proteine nötig sind, damit der Wurm berührungsempfindlich wird. Diese Proteine – einschließlich DRASIC – sind alle Mitglieder der Deg/Enac Kanal Großfamilie. Aus dieser Kenntnis leitet sich die Idee ab, dass mechanische Kräfte, die auf die Membran wirken, zur Öffnung des Kanals führen, der auf diese Weise ein mechanischer Sensor würde. Derselbe Kanal hilft offenbar sensorischen Neuronen, harmlose und schmerzhafte Reize auf vollständig verschiedene Weise zu registrieren. Es ist bekannt, dass DRASIC in der Lage ist, heteromere Kanalkomplexe mit anderen Mitgliedern der Deg/Enac Familie zu bilden.

Ein weiterer Aspekt der Funktion des DRASIC Kanals steckt in der Tatsache, dass eine Zunahme des Säuregrads – also das Auftreten von Protonen – als effektiver Reiz zur Kanalöffnung funktioniert. Die Zunahme des Säuregrads ist die universelle Konsequenz einer Verletzung oder Entzündung des peripheren Gewebes. Mäuse, die nicht über DRASIC verfügten, waren jedoch viel weniger durch die Muskelsäuerung betroffen. Mit anderen Worten, die Abwesenheit des Kanalproteins hatte einen analgetischen Effekt. Die Verschiedenheit der Funktionen für den DRASIC Kanal, die hier zum ersten Mal vorgestellt wird, zeigt direkt, dass der Kanal nicht allein, sondern im Verbund mit anderen Proteinen funktioniert, die bislang unbekannt sind. Es ist nun genau diese Art der in vivo Analyse einer Proteinfunktion, die das Prinzip deutlich macht, demzufolge die Funktion eines einzelnen Proteins je nach dem zellulären Kontext enorm variieren kann. Produkte einzelner Gene sind also nicht bloß einfache Einheiten, die alleine operieren, um wohldefinierte Funktionen zu erfüllen. Sie üben ihre Aufgaben vielmehr in einer Vielzahl von Wegen aus, die von anderen Proteinen abhängen, mit denen sie in Wechselwirkung treten können. Weitere Details siehe Arbeitsgruppe von *Gary Lewin*.

MDC-Neujahrsveranstaltung am 28. Januar 2000: Prof. Fritz Melchers (Mitte), der frühere Vorsitzende des Wissenschaftlichen Ausschusses des MDC (Basel, Schweiz, Mitte) erhält für seine Verdienste um das MDC von Prof. Detlev Ganten (MDC-Stiftungsvorstand) (re.) die Max-Delbrück-Medaille. Interessierter Zuschauer: Wolf-Michael Catenhusen (Kuratoriumsvorsitzender des MDC und Parlamentarischer Staatssekretär im Bundesforschungsministerium).
At the MDC`s New Year Reception on January 28, 2000: Prof Fritz Melchers (in the middle), former chairman of the Scientific Committee of the MDC from Basle (Switzerland) receives the Max Delbrück Medal for his contributions to the development of the MDC from MDC`s Scientific Director, Detlev Ganten (MD., Ph.D; right) as Wolf-Michael Catenhusen, chairman of the MDC`s Board of Trustees and Parliamentary State Secretary from the Federal Ministry for Education and Research looks on.

The interdisciplinary nature of pure research is reflected in the fact that its results are of importance and have applications in almost all classic clinical disciplines. The same molecules involved in cell differentiation, growth factors, and ion channels in membranes also play important roles in cancer, cardiovascular and nervous system diseases. Molecular medicine overcomes the traditional boundaries of these disciplines and provides conditions for true content-based and structural interdisciplinary activities.

At the start of the 1980s, people began to understand for the first time how the tools of gene technology could be used to map the human genes, but for a long time many scientists were unable to make any sense of the immense efforts associated with the identification of the human gene sequence and the three billion or so building stones involved. The hope of a genetic analysis of diseases was based more on optimism than any empirical evidence. Only when clinical observations in combination with molecular biology research clearly showed that cancer, for example, is a genetic disease and the formation of tumors is affected by DNA variants, did the project really get underway, leading to the sequencing of the human genome and other model genomes. Due to these genome projects the ideas of molecular medicine became accepted by the general public with their promise of sweeping perspectives for the future.

The 1990s saw the start of a new form of collaboration in medicine whereby scientists involved in molecular genetics and genome research, using genetic engineering, tried to answer clinically relevant questions and develop new products for the healthcare market. Today, it is perhaps possible to think of the past decade as the first period of biomedicine, during which the scope of gene diagnosis was enormously extended as well as the identification of many new targets as starting points for the development of drugs of the future. These developments are not only characterized by immense

Die Interdisziplinarität in der Grundlagenforschung spiegelte sich wider in der Tatsache, dass die Ergebnisse Bedeutung haben und Anwendung finden in fast allen klassischen klinischen Fächern. Die gleichen Moleküle der Zelldifferenzierung, Wachstumsfaktoren, Ionenkanäle in den Membranen sind für Krebs, Herzkreislauf- und Nervenerkrankungen in gleicher Weise wichtig. Die Molekulare Medizin überwindet damit die traditionellen Grenzen der Disziplinen und schafft die Voraussetzung für wahrhaft inhaltliche und strukturelle Interdisziplinarität.

Zu Beginn der achtziger Jahre war zum ersten Mal verstanden worden, wie die Werkzeuge der Gentechnik es erlaubten, Genkarten des Menschen anzufertigen, aber lange Zeit hindurch konnten viele Wissenschaftler keinen Sinn in dem ungeheuren Aufwand erblicken, der mit der Erstellung der menschlichen Gensequenz und ihren rund drei Milliarden Bausteinen verbunden sein würde. Die Hoffnung auf genetische Analysen von Krankheiten beruhte mehr auf Optimismus als auf empirischer Evidenz. Erst als klinische Beobachtungen in Verbindung mit molekularbiologischen Forschungen deutlich vor Augen führten, dass zum Beispiel Krebs eine genetische Krankheit ist und die Bildung von Tumoren von DNA Varianten beeinflusst wird, kam das Projekt in Schwung, in dessen Verlauf das humane Genom und andere Modellgenome sequenziert wurden. Mit diesen Genomprojekten konnte sich die Idee der Molekularen Medizin öffentliche Anerkennung verschaffen und umfassende Perspektiven für die Zukunft bieten.

In den neunziger Jahren hat dann zunehmend eine neue Form der Zusammenarbeit auf dem Gebiet der Medizin begonnen. Dabei versuchen molekulargenetisch und genomisch orientierte Wissenschaftler mit gentechnischen Methoden klinisch relevante Fragestellungen zu beantworten und ihre Lösungen in neue Produkte für den Gesundheitsmarkt umzusetzen.

Es ist heute vielleicht möglich, vom vergangenen Jahrzehnt als einem ersten Zeitalter der Biomedizin zu sprechen, in dem nicht nur das Spektrum der Gendiagnostik enorm erweitert wurde, sondern in dem auch viele neue Zielstrukturen (Targets) erkannt wurden, die als Ausgangspunkt für die Entwicklung künftiger Medikamente dienen. Diese Entwicklung ist nicht nur durch ihre ungeheure Dynamik und das konsequent interdisziplinäre Vorgehen der Wissenschaft charakterisiert, sondern auch durch die nahtlose Zusammenarbeit von Grundlagenforschung, Klinik und Industrie, die – so lässt sich vorhersagen – in Zukunft noch intensiviert wird. Der Campus in Berlin-Buch hat von Anfang an diese Entwicklung wahrgenommen, mitgeprägt, zu ihr beigetragen und sein Vorgehen an diesem wissenschaftlichen, gesellschaftlichen und ökonomischen Verbund orientiert.

Das MDC mit seinen mehr als 700 Mitarbeiterinnen und Mitarbeitern, von denen 300 wissenschaftlich tätig sind, konzentriert seine Forschungen auf insgesamt sechs Felder:
- Herz-Kreislaufforschung,
- Krebsforschung,
- Neurowissenschaften,
- Genetik und Genomforschung, Strukturbiologie, Bioinformatik,
- Zellwachstum und Zelldifferenzierung
- Molekulare Therapie.

The multitalented molecules of molecular medicine

C/EBPs flip a switch in chromatin remodelling and gene activation

The human genome contains over 30,000 different genes. Expression of various combinations of these genes gives rise to specific cell types, such as muscle, skin, blood or nerves, and also determines whether cells remain in a resting state, grow and proliferate or die and self-destruct. Inappropriate gene regulation causes autoimmune diseases, a variety of other genetic diseases and cancer. Researchers in the MDC laboratory directed by *Achim Leutz* are interested in how cells make decisions to differentiate and how these processes are linked to the development of disease, especially leukemia. By focusing on a family of transcription factors called C/EBP proteins, which were originally identified as regulators of liver-specific genes but which are now known to be important regulators for many cell types, the MDC scientists have found that the ability of C/EBP proteins to influence various programs of differentiation depends on their ability to produce changes in chromatin structure. Chromatin is the higher order structure of chromosomes in a cell that helps keep some genes inaccessible and inactive, while others are exposed and activated. The C/EBP proteins work in concert with other transcription factors to change the structure of chromatin, in essence allowing some genes to switch from an inactive state to an activated one. The MDC research group has shown that abrogation of the interaction between C/EBP and collaborating transcription factors, such as c-Myb, is an important event during leukemogenesis. Meanwhile, workers in other laboratories have identified frequent mutations in C/EBP genes in patients with several hematological disorders. Researchers at the MDC believe that C/EBP proteins can integrate signals elicited by nutrients or growth factors, translating them into changes in chromatin structure, gene regulation and cell fate. As a key regulator of cellular differentiation, and an important contributor to oncogenesis, C/EBP proteins are ideal candidates for the development of novel therapeutic agents.

Die vielseitigen Moleküle der Molekularen Medizin

C/EBP Proteine: Schalter der Chromatinumordnung und Genaktivierung

Das menschliche Genom enthält mehr als 30.000 verschiedene Gene. Die Expression von verschiedenen Kombinationen dieser Gene führt nicht nur zu spezifischen Zelltypen wie etwa Muskel-, Haut-, Blut- und Nervenzellen, sondern bestimmt auch, ob Zellen in einem Ruhezustand bleiben oder ob sie wachsen, sich teilen, sterben und selbst zerstören. Die ungeeignete Regulation von Genen kann zu Autoimmunkrankheiten und einer Reihe von anderen Genkrankheiten bis hin zu Krebs führen. Das von *Achim Leutz* geleitete MDC-Laboratorium ist an der Frage interessiert, wie Zellen Entscheidungen bei der Differenzierung treffen und wie diese Vorgänge mit dem Entstehen von Krankheiten verbunden sind, wobei das besondere Augenmerk der Leukämie gilt. Indem sie sich auf eine Familie von Transkriptionsfaktoren mit Namen C/EBP Proteinen konzentriert haben, die ursprünglich als Regulatoren von leberspezifischen Genen identifiziert worden waren und von denen man inzwischen weiß, dass sie wichtige Regulatoren für viele Zelltypen sind, haben die Wissenschaftler am MDC herausfinden können, dass die Fähigkeit der C/EBP Proteine, verschiedene Differenzierungsprogramme zu beeinflussen, auf ihrem Vermögen beruht, Änderungen der Chromatinstruktur zu bewirken. Chromatin heißt die höhere Ordnungsstruktur der Chromosomen in einer Zelle, mit deren Hilfe einige Gene unzugänglich und inaktiv gehalten werden, während andere exponiert und aktiviert werden. Die C/EBP Proteine arbeiten mit anderen Transkriptionsfaktoren zusammen, um die Struktur des Chromatins zu ändern, wobei vor allem einige Gene ihren inaktiven Zustand verlassen und aktiv werden. Die MDC Forschungsgruppe hat gezeigt, daß die Aufhebung der Wechselwirkung zwischen den C/EBP Proteinen und anderen Transkriptionsfaktoren wie etwa c-Myb ein wichtiger Schritt während der Entstehung der Leukämie ist. In der Zwischenzeit haben andere Laboratorien häufige Mutationen in C/EBP Genen in Patienten mit mehreren hämatologischen Störungen identifiziert. Forscher am MDC glauben, daß C/EBP Proteine Signale integrieren könnten, die von Nährstoffen oder Wachstumsfaktoren stammen, um sie in Änderungen der Chromatinstruktur, der Genregulation und des Schicksals einer Zelle umzusetzen. Als Schlüsselmoleküle der Zelldifferenzierung und als wichtige Faktoren der Onkogenese sind die C/EBP Proteine ideale Kandidaten für die Entwicklung neuartiger therapeutischer Substanzen.

dynamic and subsequent interdisciplinary scientific activities but also by the ready collaboration between pure research, the clinics and industry, which – as previously said – will be even more intense in the future. Right from the start, the Berlin-Buch Campus has recognized the importance of this and been an enthusiastic participant by adopting a perspective that combines scientific, social and economic attitudes.

The MDC, with over 700 staff, 300 of whom are engaged in scientific work, is concentrating its research efforts in six areas:

- Cardiovascular research,
- Cancer research,
- Neurosciences,
- Genetics and Genome Research, Structural-Biology, Bioinformatics,
- Cell Growth and Cell Differentiation
- Molecular Therapy.

These are described in detail in this Research Report. The Forschungsinstitut für Molekulare Pharmakologie with almost 200 staff is collaborating closely with the MDC in its search for targets for new drugs. They are investigating the three-dimensional structure of biological macromolecules and using this information to characterize their pharmacolog-

Die Schwerpunkte werden in diesem Forschungsbericht im Detail vorgestellt.

Das Forschungsinstitut für Molekulare Pharmakologie (FMP) mit seinen knapp 200 Mitarbeiterinnen und Mitarbeitern, mit dem das MDC eng zusammenarbeitet, fahndet nach Zielpunkten für neue Medikamente. Man versucht, biologische Makromoleküle unter dieser Vorgabe in ihrer Raumstruktur aufzuklären und auf pharmakologische Wirkungen hin zu charakterisieren, ohne dabei die komplexe Biologie einer Zelle aus den Augen zu verlieren.

Die Besonderheit des MDC besteht in der Orientierung seiner Forschungen an den klinischen Fragestellungen, und die beiden Kliniken, mit denen die Wissenschaftler kooperieren, sind die Robert-Rössle-Krebs-Klinik und die Franz-Volhard-Herz-Kreislauf-Klinik.

Oben wurde der Hinweis gegeben, dass das Humane Genomprojekt unter anderem deshalb in die Wege geleitet wurde, weil erkannt worden war, dass zahlreiche genetische Komponenten zur Entwicklung von Krebs beitragen können. Inzwischen setzt sich die wissenschaftlich begründete Ansicht durch, dass es für eine Behandlung besser ist, einen Tumor (Krebsgeschwulst) nicht mehr nur durch das Organ zu

ical effects, without losing sight of the complex biological processes that take place within cells.
The uniqueness of the MDC lies in the application of its research efforts to answer clinical questions, and the two clinics with which the researchers collaborate are the Robert Rössle Cancer Center and the Franz Volhard Clinic for Cardiovascular Diseases.

It was mentioned earlier that the Human Genome Project, due to the way it was conducted, showed that many genetic components could contribute to the development of cancer. Since then, researchers have found that it is better for treatment if a tumor is no longer just characterized by the organ in which it occurs. It is much more desirable to carry out a precise analysis of as many genes as possible in the cancerous transformed cells and associated tissues, as is possible today with gene chips. Now scientists talk about the genetic profile of a tumor and they are able to use this to develop more specific treatments which are based on particular situations.
The application of molecular medicine has a number of advantages, such as a better choice of available drugs, since a genetic analysis can show which drugs have too narrow a spectrum of activity or highlight cases where undesirable side effects are likely to occur. Similar developments are expected in the treatment of cardiovascular diseases, if researchers manage to obtain more detailed information on the genetic causes of cardiomyopathies or learn more about the molecular mechanisms which lead to high blood pressure or heart failure.
These problems are the subject of intensive study at the MDC where pure scientists have to rely on simplified systems, models and animal experiments because of the complexity of the problems. In our laboratories genes have been identified which in altered (mutated) form can trigger changes in heart function, blood pressure regulation or the nervous system which clinicians see everyday in their patients.

charakterisieren, in dem er entstanden ist. Vielmehr ist es geboten, in den krebsartig transformierten Zellen und dem erkrankten Gewebe eine genaue Analyse von möglichst vielen Genen durchzuführen, wie sie heute mit sogenannten Genchips gelingt. Man spricht dabei von dem genetischen Profil eines Tumors und kann mit seiner Hilfe das Ziel einer Therapie in Angriff nehmen, die der individuellen Situation angemessen und insofern massgeschneidert ist.

Der Ansatz der Molekularen Medizin erlaubt unter anderem eine bessere Auswahl unter den verfügbaren Medikamenten, da eine Genanalyse Hinweise gibt, welche Arzneistoffe über eine zu geringe Wirksamkeit verfügen bzw. in welchen Fällen unzumutbare Nebenwirkungen auftreten können. Ähnliche Entwicklungen sind für die Behandlung von Herz-Kreislauferkrankungen zu erwarten, wenn es gelingt, die genetischen Ursachen etwa von Kardiomyopathien genauer zu verstehen oder mehr von den molekularen Mechanismen zu erfahren, die Bluthochdruck- oder eine Herzinsuffizienz zur Folge haben.

Am MDC wird intensiv an diesen Fragen gearbeitet, wobei die Grundlagenforschung wegen der Komplexität der Fragestellungen auf reduktionistische Systeme, Modelle und Tierversuche angewiesen bleibt. Dabei konnten in unseren Laboratorien Gene identifiziert werden, die in veränderter (mutierter) Form Störungen bei der Herzfunktion, Blutdruckregulation oder im Nervensystem auslösen, wie sie den Klinikern auch bei erkrankten Menschen bekannt sind.

Wenn die Forscher des MDC in Zusammenarbeit mit den Kliniken erforschen, wie es möglich wird (und hoffentlich zu verhindern ist), dass aus einer Genvariante eine krankhafte Störung – Krebswachstum oder Herzkreislaufkrankheit – wird, dann legen sie ihren Überlegungen ein Konzept zugrunde, das sich zum ersten Mal bei Max Delbrück findet. Es geht um die Idee der Signalumwandlung bzw. der Signalkette und

A simple blood test may increase patient safety *before* gene therapy with adenoviruses

Adenoviruses are the most widely used vectors in gene therapy studies and their role is to transport the therapeutic genes to their site of action. Viruses are particularly versatile and effective gene transporters. There are great expectations that they will useful in the treatrment of cancer. However, two years ago, the 18-year-old Jesse Gelsinger died suddenly in the USA from organ failure after he had received an injection of a genetically modified adenovirus, used as a gene taxi, directly into his bloodstream. The exact molecular biological cause of his death remains unknown. However, new information about a previously unknown immune reaction to adenoviruses, or as “Science” commented on January 25, 2002, “a new piece in the puzzle”, has now come from a research team led by *Günter Cichon* from the Humboldt University, Berlin, at the Max Delbrück Center for Molecular Medicine (MDC) and *Reinhard Burger* from the Robert Koch Institute, Berlin, and published in the journal “Gene Therapy” (Vol. 8 (2001), pp. 1794-1800). Under laboratory conditions, high concentrations of the adenoviruses used in gene therapy produce an unexpected intense activation of the so-called complement system. The complement system consists of a group of proteins which circulate in the blood and act as an initial defense mechanism against an assault by infectious pathogens. In order to increase patient safety, the authors suggest carrying out a simple blood test to measure the degree of the complement reaction before gene therapy.
More detailed information can be obtained in the report of the Humboldt University research group at the MDC.

Ein einfacher Bluttest könnte die Sicherheit der Patienten *vor* einer Gentherapie mit Adenoviren erhöhen

Adenoviren sind die am meisten benutzten Vektoren bei Gentherapie-Studien. Ihre Aufgabe ist es, therapeutische Gene an ihren Wirkort zu transportieren. Viren sind besonders vielseitige und wirksame Gentaxis. Große Hoffnungen, z. B. bei der Behandlung von Krebs, gründen sich auf deren Einsatz. Vor zwei Jahren starb jedoch plötzlich der 18-jährige Jesse Gelsinger in den USA an Organversagen, nachdem er eine Injektion eines genetisch veränderten, als Gentaxi benutzten Adenovirus direkt in den Blutstrom erhalten hatte. Die genaue molekularbiologische Ursache seines Todes blieb bisher rätselhaft. Neue Informationen über eine zuvor unbekannte Immunreaktion gegenüber Adenoviren, oder wie die „Science“ am 25. Januar 2002 kommentierte „ein neues Stück des Puzzles“ haben nun ein Team um *Günter Cichon* von der Humboldt-Universität zu Berlin am Max-Delbrück-Centrum für Molekulare Medizin (MDC) und *Reinhard Burger* vom Robert-Koch-Institut Berlin vorgelegt und in der Zeitschrift „Gene Therapy“ (Band 8 (2001), pp. 1794–1800) publiziert. Unter Laborbedingungen können die bei der Gentherapie verwendeten hohen Konzentrationen an Adenoviren eine unerwartet heftige Aktivierung des so genannten Komplementsystems hervorrufen. Dieses System besteht aus einer Gruppe von Proteinen, die im Blut zirkulieren und gemeinsam infektiöse Pathogene angreifen. Um die Sicherheit von Patienten zu erhöhen, schlagen die Autoren vor, den Grad der Komplementreaktion künftig vor einer Gentherapie mit einem einfachen Bluttest zu messen. Weitere Einzelheiten im Bericht der Forschungsgruppe der Humboldt Universität am MDC.

If MDC researchers work in close collaboration with the clinics to discover how it is possible (and hopefully how it can be prevented) that a gene variant can cause a serious change – the growth of a tumor or cardiovascular disease – then their conjectures will form the basis of a concept that was first proposed by Max Delbrück. This involves the idea of signal transformation or the signal chain and signal transduction. In this context, Delbrück meant the molecular route that led from an altered gene sequence via the changed gene product (protein), then via another intermediate information carrier to the final result which manifested itself as a disease. As far as Delbrück was concerned, a mechanism could only be understood if the route of signal transfer was completely known. This has often led to a number of major surprises, such as just how many different effects a signal molecule can exhibit. One example of this is "β-catenin", which appears to have a key function in the life of cells.

Signaltransduktion. Delbrück meinte damit den molekularen Weg, der von einer veränderten Gensequenz über das dann veränderte Genprodukt (Protein) und weiter über andere Zwischenträger der Information bis zum Endergebnis führt, das sich als Krankheit zeigt. Für Delbrück war ein Mechanismus erst dann verstanden, wenn sich der Signalübertragungsweg lückenlos nachvollziehen ließ. Dabei treten oft große Überraschungen zutage, nämlich dann, wenn sich herausstellt, wie vielfältig einige Signalmoleküle sein können. Ein Beispiel hierfür ist das „Beta-Catenin", das im Leben von Zellen eine Schlüsselfunktion zu haben scheint.

Unter dem oben genannten Gesichtspunkt der Signalumwandlung lassen sich auch neurobiologische Arbeiten aus dem MDC anführen, in denen es nicht nur wie früher schon bei den Arbeiten von Timoféeff-Ressovsky um die Neurogenese – die Entwicklung des Nervensystems – geht, sondern mit denen die Mechanismen erkundet werden, die dem Ab-

The multitalented molecules of molecular medicine

One single signal molecule controls the programs responsible for the development of both hair and skin

Adult skin stem cells are able to produce two different types of cells: they can develop into skin cells of the outer layer of the skin (epidermal cells) as well as hair follicles with the horn cells of the hair. MDC researchers Walter Birchmeier and Jörg Hülsken have now discovered the signal that drives the development potential of skin stem cells in the direction of the skin as well as the signal that drives them in the direction of hair. The answer is a surprising one in that the choice of direction involves a single signal molecule, β-catenin. This key molecule is well known to Walter Birchmeier´s group since it plays a central role in an important cell communication system, the Wnt/β-catenin signal pathway. For years the group has been studying this signal pathway which controls the transfer of signals from the cell surface to its nucleus. For some time researchers have believed that β-catenin must be involved in the formation of skin and hair. β-Catenin plays a role in a type of tissue related to hair, the feathers of birds. In addition, according to Birchmeier, mutations in other components of the Wnt-pathway in the mouse have provided "certain information" about defects in teeth, mammary glands and hair. However, absolutely nothing was known about which cells were involved and how β-catenin worked. It was mice, which had been modified by Jörg Hülsken so that only the β-catenin gene in their skin had been switched off (so-called conditional knock-out), that provided the explanation: these mice lost their hair 20 to 30 days after birth. They then remained naked, because they were unable to produce new hair follicles which would have led to the growth of new hair. In the adult, healthy animal the hair follicles undergo a continuous process of self-renewal, which is triggered by stem cells producing hair follicles. Only then can the hair continue to grow. In addition, it was found that knock-out mice were not just bald: the skin of these animals contained unusual cysts coated with stem cells. These cysts were produced by a defect in stem cell regulation, which resulted in stem cells losing their ability to form hair follicle cells. Therefore, the results obtained by the MDC researchers showed two things: 1. Skin stem cells require β-catenin, in order to produce hair follicles. 2. If there is no β–catenin available, then the stem cells cannot produce hair cells, only skin cells. The fact that β-catenin controls the development program for stem cells is a new discovery. Investigations by other groups have shown that the Wnt/β-catenin signal pathway also plays an important role in the control of other stem cells (e.g. the Lieberkühn crypts in the small intestine).

For further details see research group of *Walter Birchmeier*

Die vielseitigen Moleküle der Molekularen Medizin

Ein einziges Signalmolekül steuert die Entwicklungsprogramme für Haut und Haare

Adulte Stammzellen der Haut haben die Fähigkeit, zwei verschiedene Zelltypen auszubilden: Aus ihnen können sowohl Hautzellen der Oberhaut (epidermale Zellen) als auch Haarfollikel mit den Hornzellen der Haare hervorgehen. Welche Signale dieses Entwicklungspotenzial der Hautstammzellen einmal in Richtung Haut und einmal in Richtung Haar regulieren, haben Wissenschaftler des MDC um Walter Birchmeier und Jörg Hülsken herausgefunden. Dabei stellte sich überraschenderweise heraus, dass für die Wahl der Richtung nur ein einziges Signalmolekül, das β-Catenin, ausschlaggebend ist. Dieses Schlüsselmolekül ist ein alter Bekannter von Walter Birchmeier´s Gruppe, da es eine zentrale Rolle in einem wichtigen Kommunikationssystem der Zelle, dem Wnt/β-Catenin-Signalweg einnimmt. Zur Kenntnis dieses Signalweges, der dafür sorgt, dass Signale von der Oberfläche der Zelle in den Zellkern gelangen, hat die Gruppe seit Jahren wesentlich beigetragen. Schon seit einiger Zeit hatte man daher vermutet, dass β-Catenin in die Haut- und Haarbildung eingebunden sein muss. So spielt β-Catenin bei einem dem Haar verwandten Organ, den Federn von Vögeln, eine Rolle. Auch Mutationen in anderen Komponenten des Wnt-Weges in der Maus haben, so Birchmeier, „gewisse Hinweise" auf Defekte in Zähnen, den Brustdrüsen und eben in den Haaren ergeben. Völlig unbekannt war jedoch, in welchen Zellen und auf welche Weise β-Catenin wirkt. Erst Mäuse, bei denen durch Jörg Hülsken das β-Catenin-Gen nur in der Haut ausgeschaltet wurde (sogenannter konditionaler knock-out), lieferten eine Erklärung: solche Mäuse verloren 20 bis 30 Tage nach der Geburt ihr Haarkleid. Danach blieben sie nackt, weil sich keine neuen Haarfollikel mehr bilden und damit auch kein Haar mehr nachwachsen konnte. Im erwachsenen, gesunden Organismus sind die Haarfollikel einem ständigen Selbsterneuerungsprozess unterworfen, der durch Stammzellen an den Seiten der Haarfollikel ausgelöst wird. Nur dann kann das Haar ständig nachwachsen. Weiterhin wurde festgestellt, dass Knock-out-Mäuse nicht bloß kahl waren: in der Haut der Tiere fanden sich ungewöhnliche, mit Hautzellen ausgekleidete Zysten. Diese Zysten entstehen aus fehlgeleiteten Stammzellen, die ihre Fähigkeit verloren haben, Haarfollikelzellen zu bilden. Mit ihren Forschungen konnten die MDC-Forscher demnach zweierlei zeigen: 1. Stammzellen der Haut benötigen β-Catenin, um Haarfollikel zu bilden. 2. Fehlt β-Catenin, so bilden sich aus den Stammzellen keine Haare sondern lediglich Hautzellen. Die Tatsache, dass β-Catenin die Entwicklungsprogramme von Stammzellen steuert, ist eine neue Erkenntnis. Untersuchungen anderer Gruppen haben ergeben, dass der Wnt/β-Catenin-Signalweg auch bei der Kontrolle anderer Stammzellen (z. B. der Lieberkühn-Krypten im Dünndarm) eine wichtige Rolle spielt.

Weitere Details siehe Arbeitsgruppe *Walter Birchmeier*

From the above viewpoint of signal transfer, one can also cite neurobiological studies from the MDC, not only the early work involving the research of Timoféev-Ressovsky on neurogenesis – the development of the nervous system – but also on how those mechanisms are signaled, which are fundamental for the death of brain tissue. For example, at the MDC, researchers are investigating the signals that are responsible for the stability of the connections (synapses) between neurons or initiate their destruction. Scientists are also carrying out research on glial cells and neurons and the sites where the signals manifest their effects (receptors), as well as the genes that are involved in the production of the relevant proteins.

The entrepreneurial side

Alongside these research facilities and clinics, over forty biotech-oriented companies have set themselves up in Berlin-Buch using the Campus as a biotechnology park and, together with the associated service companies and supply firms, they employ over 550 staff. These entrepreneurial activities are coordinated by "BBB Management GmbH Campus Berlin Buch" which was set up in 1995. Since 1998, it has operated an Innovation and Start-up Center (German abbrev. IGZ), the second stage of which was completed in Spring 2001. With its help, it should be easier for scientists to transfer the results of their basic research into useful applications and assist in the commercial exploitation of their business ideas. The biotech companies, which are located on the Buch Campus offer a variety of new techniques, for example, to improve the diagnosis of myocardial infarction; they are also trying to link the vast synthetic ability of micro-organisms with modern

sterben von Hirngewebe zugrunde liegen. Gefragt wird am MDC konkret zum Beispiel nach den Signalen, die für die Stabilität der Verbindungen (Synapsen) zwischen Neuronen sorgen oder ihren Abbau in die Wege leiten und wie sich Synapsen bei der Entwicklung des Gehirns entfalten. Erforscht wird auch die Funktion von Gliazellen und Neuronen und ihren Rezeptoren. Weiter wird erforscht, welche Gene an der Herstellung der entsprechenden Proteine beteiligt sind.

Die unternehmerische Seite

Neben den genannten Forschungseinrichtungen und den Kliniken haben sich in Berlin-Buch mehr als vierzig biotechnisch ausgerichtete Firmen eingerichtet, die den Campus als Biotechnologiepark nutzen und zusammen mit dazugehörigen Dienstleistungsunternehmen und Zulieferfirmen mehr als 550 Angestellte haben. Koordiniert werden die unternehmerischen Aktivitäten durch die BBB Management GmbH, die 1995 gegründet worden ist. Sie betreibt seit 1998 ein Innovations- und Gründerzentrum (IGZ), dessen zweite Baustufe im Frühjahr 2001 fertig geworden ist. Mit seiner Hilfe soll es für Wissenschaftler leichter werden, Ergebnisse aus der Grundlagenforschung in verwertbare Anwendungen zu überführen und ihrer Geschäftsidee den Weg auf den Markt zu ermöglichen (Existenzgründungen). Die Biotechfirmen, die auf dem Campus in Buch vertreten sind, bieten unter anderem neue Verfahren an, um Herzinfarkte besser diagnostizieren zu können; sie versuchen, die vielfältigen Syntheseleistungen von Mikroorganismen mit den modernen Methoden der Genomanalyse zu verbinden, um rascher Zugang zu bioaktiven Substanzen und damit zu neuen Medikamenten zu bekom-

The multitalented molecules of molecular medicine

Cholesterol in the brain: Making or breaking synapses

"The smooth operation of the nervous system depends on rapid communication between nerve cells at meeting areas called synapses. Although synapses were first identified 100 years ago, their formation, a process called synaptogenesis, has remained something of a mystery. For example, it is still not clear how many synapses a neuron can make with other nerve cells. Is the number of synapses rigidly preprogrammed or is it fluid, governed by interactions with neighboring cells? Thanks to a simple model system in which a defined type of neuron from the central nervous system is purified and cultured, two intriguing but unanticipated conclusions about synaptogenesis have been reached. The first is that neurons by themselves form few synapses unless they have help from other nerve cells called glial cells, the second is that the synapse-promoting signal released by glial cells is cholesterol." Cholesterol, hitherto known as an essential neutral lipid constituent, makes its "nervous debut".

"These studies suggest that glial cells, long thought to be passive bystanders in the formation and operation of our neural circuitry, actively participate in the making and breaking of synapses."

Excerpted from a comment of B.A. Barres and S.J. Smith (Stanford University) to papers from Pfrieger´s group at the MDC - SCIENCE 294 (2001), 1296-97

So, cholesterol is not only of interest to cardiovascular researchers because of the way it coats arteries, it is also being studied by neurologists. Further details of this are given in the report of the work of *Frank Pfrieger* and his group.

Die vielseitigen Moleküle der Molekularen Medizin

Das Cholesterol im Gehirn: Aufbau oder Abbau der Nervenübertragung

„Das reibungslose Operieren des Nervensystems hängt von der raschen Kommunikation zwischen Nervenzellen ab, die sich an Orten begegnen, die Synapsen heißen. Obwohl Synapsen zum ersten Mal vor 100 Jahren identifiziert wurden, stellen ihre Formation und der dazugehörige Prozeß der Synaptogenese nach wie vor ein Geheimnis dar. So ist zum Beispiel nicht klar, wie viele Synapsen ein Neuron mit anderen Nervenzellen ausbilden kann. Ist die Zahl der Synapsen starr programmiert, oder ist sie flexibel in Abhängigkeit von der Wechselwirkung mit benachbarten Zellen? Dank eines einfachen Modellsystems, in dem eine bestimmte Art von Neuron aus dem zentralen Nervensystem gereinigt und kultiviert wird, konnten gleich zwei verblüffende und unerwartete Schlüsse über die Synaptogenese gezogen werden. Der erste besteht darin, dass Neuronen selbst nur wenige neue Synapsen ausbilden, solange sie keine Hilfe von anderen Nervenzellen namens Gliazellen bekommen, und der zweite besteht darin, dass das Signal, das Gliazellen freisetzen, um die Bildung von Synapsen anzuregen, das Cholesterin ist, das auf diese Weise sein Debüt im Nervensystem macht.

„Diese Experimente legen den Gedanken nah, daß die Gliazellen, die man lange als passive Zuschauer sowohl bei der Bildung von Synapsen als auch bei der Operation des Nervensystems angesehen hat, sich vielmehr aktiv am Auf- und Abbau der Synapsen beteiligen."

Auszüge aus einem Kommentar von B.A. Barres und S.J. Smith (Stanford Universität) zu Arbeiten der Pfrieger Gruppe vom MDC: SCIENCE 294 (2001), 1296–97

Cholesterin interessiert also nicht nur die Herz-Kreislaufforscher bezüglich der Entstehung von Arterienverkalkung, sondern auch die Hirnforscher. Näheres im Bericht der Arbeitsgruppe von *Frank Pfrieger.*

methods of genome analysis in order to obtain more rapid access to bioactive agents, leading eventually to new drugs; they are also involved in the development of new types of agents which can be used in cancer therapy, and drugs which can modulate the immune system so that the body's defensive reaction after organ transplantation is reduced to a minimum; in addition, they offer tools for the reliable sequencing of DNA-fragments on a large scale, in order to produce biomedically relevant genes and establish the associated risk profile or to predict both the beneficial and side effects of drugs (Pharmacogenetics).

Many of the firms have been able to obtain venture capital in recent years in order to enter into an expansive phase of development. The resulting demand for laboratory and office space cannot be met in the long-term by the Campus itself. Dealing with requests from people from other regions at home and abroad to come to Berlin-Buch, is the responsibility of BBB Management GmbH, which is currently closely involved in expanding the Biotechnology Park beyond the Campus itself.

When scientists become involved in medical problems, a discovery can be made in the laboratory at any time which can have long-term commercial implications. Scientific institutions must, therefore, think about exploiting their research findings (technology transfer). In collaboration with the other Helmholtz Research Centers, the GBF (Braunschweig), the GSF (Munich) and the DKFZ (Heidelberg), the MDC has set up a company called Ascenion, whose task it is to undertake the centralized marketing of the results of the biomedically oriented research institutions that belong to the Helmholtz-Association of German Research Centers. Any income from these activities will be ploughed back into the participating research institutes and research groups or to those responsible for the discoveries.
The background to this coordinated activity is the increasing sensitivity of scientists regarding the potential economic applications of their research results and this is reflected in the increasing number of patent applications and start-up companies. This also includes the MDC.

The public understanding of science

The future of molecular medicine does not just depend on the results of research and their associated application in the clinics and in general practice. It is also determined by the response to the questions: "How much do the general public understand about modern biomedicine and genetics?" and "How well informed do individual citizens feel?" Patient education will be an important "drug" in the future.

As far as these questions are concerned, the Campus is trying to help in two ways. On the one hand, a Life Science Learning Laboratory has been built where, on a daily basis, school children, their teachers and other groups are taught about the elementary methods which are needed to understand genetics, gene technology and genome research. The Life Science Learning Laboratory is currently being extended with financial support from the European Regional Development Fund (German abbrev. EFRE). On the other hand, the MDC – also within

men; sie bemühen sich um die Entwicklung von neuartigen Substanzen, die zur Krebstherapie eingesetzt werden können, und von Arzneien, die das Immunsystem so modulieren, dass die Abwehrreaktion nach Organtransplantationen minimal wird; sie bieten die verlässliche Sequenzierung von DNA-Abschnitten in großem Maßstab an, um biomedizinisch relevante Gene und dazugehörige Risikoprofile zu erstellen oder um die Wirkungen und Nebenwirkungen von Medikamenten vorherzusagen (Pharmakogenetik).

Die zwischen 1998 und 2001 errichteten neuen Laborgebäude des Biotechnologieparks.
The new laboratory buildings in the Biotechnology Park erected between 1998 and 2001.

Mehrere der Unternehmen konnten in den vergangenen Jahren erfolgreich Risikokapital („venture capital") einwerben und in eine expansive Phase der Entwicklung eintreten. Die daraus resultierende Nachfrage nach Labor- und Bürofläche kann langfristig auf dem Campusareal selbst nicht mehr gelöst werden. Die Nachfrage nach Ansiedlungsmöglichkeiten, die aus anderen Bundesländern und dem Ausland in Berlin-Buch eintreffen, veranlasst die BBB Management GmbH, sich daher zur Zeit intensiv um eine Erweiterung des Biotechnologieparks über den Campus hinaus zu bemühen.

Wenn sich Grundlagenforscher medizinischen Fragestellungen zuwenden, kann jederzeit im Laboratorium eine Entdeckung gemacht werden, die sich langfristig vermarkten lässt. Wissenschaftliche Institutionen müssen sich daher rechtzeitig Gedanken um die Verwertung von Forschungsergebnissen machen (Technologietransfer). Das MDC hat zusammen mit anderen, ebenfalls der Helmholtz-Gemeinschaft gehörenden Zentren, der GBF (Braunschweig), der GSF (München) und dem DKFZ (Heidelberg), die Firma Ascenion gegründet, deren Aufgabe die zentrale Vermarktung von Arbeitsresultaten aus biomedizinisch orientierten Forschungsinstitutionen ist, die zur Helmholtz-Gemeinschaft Deutscher Forschungszentren e. V. gehören. Eventuell erwirtschaftete Erträge werden dabei an die beteiligten Forschungseinrichtungen und Arbeitsgruppen bzw. die Erfinder zurückfließen.
Hintergrund dieser koordinierten Aktivität ist die zunehmende Sensitivität von Wissenschaftlern für eine mögliche ökonomische Umsetzung ihrer Forschungsergebnisse, die sich unter anderem an der zunehmenden Zahl von Patentanmeldungen und Existenzgründungen auch am MDC zeigt.

the context of the infrastructure of EFRE support – has obtained funding for the construction of a communications center for science (MDC.C), which was opened in Fall 2001 by the Federal President Johannes Rau on the occasion of the awarding of the President's "Prize for Technology and Innovation".

Starting in 2002, the Communications Center in association with the Berlin company, K.I.T. GmbH, holds scientific conferences and other cultural activities which are compatible with the proclaimed aims of the MDC and try to give a new dimension to the "public understanding of science". The MDC.C promotes communication both at a scientific level – by means of specialist conferences – as well as at a public level between interested groups and individual members of the public to demonstrate the role the scientific process plays in our culture and the close links between the sciences, the arts and culture. Ethical questions are also discussed. The German Research Organizations have designated 2001 as the "Year of the Life Sciences" and the MDC has been closely involved in many of the activities.

The future of molecular medicine

When, at the start of 2001, the first reliable total sequences of the human genome were presented, some scientists dared to predict that the future would be shaped by this new information in the context of a new molecular form of medicine. As far as the predictions for 2010 are concerned, these include the availability of genetic tests which can be used for the ("preventive") prediction of about a dozen diseases. This will allow prevention of diseases on an individual basis. This would also be accompanied by effective legislation which would prevent any form of genetic discrimination. The year 2020 will see the announcement of methods for the treatment of cancer which will be tailor-made to suit the genetic profile of a particular tumor and, in addition, scientists expect drugs to be tailor-made to suit individual patients, with their development and selection being based on genetic testing. Even 10 years ago, anyone who had dared to make these predictions would not have been believed. Now, they are made by successful scientists and accepted as sensible. One can understand this and, thanks to molecular medicine, they can be

Das neue Kommunikationszentrum des MDC, das Max Delbrück Communications Center (MDC.C)
MDC's new Max Delbrück Communications Center (MDC.C)
Copyright: MDC/Uwe Eising

Das öffentliche Verständnis von Wissenschaft

Die Zukunft der Molekularen Medizin wird nicht nur von Forschungsergebnissen und der dazugehörenden Umsetzung in die klinische oder allgemeine Praxis abhängen. Sie wird auch durch die Antwort auf die Frage bestimmt werden, wie groß das Verständnis in der Öffentlichkeit für die moderne Biomedizin und Genetik sein wird und wie gut informiert sich der einzelne Bürger fühlt. Die Bildung der Patienten wird ein wichtiges „Arzneimittel" der Zukunft werden.

Der Campus versucht, bei diesen Fragen auf zwei Weisen zu helfen. Auf der einen Seite wurde ein Gläsernes Labor eingerichtet, in dem täglich Schüler mit ihren Lehrern und andere Gruppen in die elementaren Methoden eingeführt werden, die zum Verständnis der Genetik, Gentechnologie und Genomforschung gehören. Das Gläserne Labor wird zur Zeit mit Unterstützung des Europäischen Fonds zur regionalen Entwicklung (EFRE) weiter ausgebaut. Auf der anderen Seite konnte das MDC – ebenfalls im Rahmen der EFRE-Infrastrukturförderung – Mittel zum Bau eines Kommunikationszentrums für die Wissenschaft einwerben (MDC.C), das im Herbst 2001 von Bundespräsident Johannes Rau im Rahmen der Verleihung des „Deutschen Zukunftspreises" eröffnet wurde.

Das Kommunikationszentrum führt in Verbindung mit der in Berlin ansässigen K.I.T. GmbH wissenschaftliche Konferenzen und andere kulturelle Veranstaltungen durch, die mit den geschilderten Zielen des MDC vereinbar sind und den Bemühungen um ein „Public Understanding of Science" eine neue Dimension geben. Das MDC.C fördert die Kommunikation sowohl auf der wissenschaftlichen Ebene – durch Fachkonferenzen – als auch im gesellschaftlichen Bereich zwischen interessierten Gruppen und einzelnen Menschen. Ethikdiskurse wird es ebenso geben wie Veranstaltungen, die zeigen, welchen Anteil das wissenschaftliche Vorgehen an unserer Kultur hat und wie eng Wissenschaft, Kunst und Kultur verbunden sind. Die Deutschen Forschungsorganisationen hatten 2001 zum „Jahr der Lebenswissenschaften" ausgerufen und das MDC hatte sich intensiv daran beteiligt.

Die Zukunft der Molekularen Medizin

Als zu Beginn des Jahres 2001 die ersten zuverlässigen Gesamtsequenzen des menschlichen Genoms vorgelegt wurden, wagten einige Wissenschaftler vorherzusagen, welche Möglichkeiten sich mit den neu gewonnenen Informationen für die Molekulare Medizin eröffnen könnten. Zu den Vorhersagen für das Jahr 2010 gehört die Verfügbarkeit von genetischen Testverfahren, die zur („präventiven") Vorhersage für rund ein Dutzend Krankheiten eingesetzt werden können. Dieses ermöglicht eine individuelle Vorbeugung (Prävention) von Krankheiten. Dazu wird begleitend zugleich eine wirksame Gesetzgebung kommen, die jedwede Form von genetischer Diskriminierung verhindert. Für das Jahr 2020 werden Methoden der Krebsbehandlung angekündigt, die an dem genetischen Profil des Tumorgewebes ausgerichtet sind, und außerdem erwarten die Wissenschaftler individualisierte – für einzelne Patienten massgeschneiderte – Medikamente, die nach genetischen Tests entwickelt und ausgewählt werden. Vor 10 Jahren wäre jeder ungläubig angesehen worden, der es

Prof. Eric S. Lander vom Whitehead Institute des Massachusetts Institute of Technology (MIT), Cambridge, USA bei seiner „Berlin Lecture“ über „The Human Genome and Beyond“ am 5. Dezember 2001 im neuen Kommunikationszentrum des MDC.
Professor Eric S. Lander (Whitehead Institute, Massachusetts Institute of Technology MIT, Cambridge, USA) delivering his “Berlin Lecture” on “The Human Genome and Beyond” on December 5, 2001 in MDC`s new Max Delbrück Communications Center (MDC.C)
Copyright: MDC; Dr. Ulrich Scheller

regarded as realistic. On the Berlin-Buch Campus we are working to make these predictions come true. The next ten years will show just how successful we will be. One thing is certain, that the medical care and human investment in the future will be more important than ever.

As far as we in Berlin-Buch are concerned, the term “molecular medicine” is more than just the application of the methods of molecular biology, gene technology and genome research to the understanding of the molecular biological mechanisms governing health and disease. We regard molecular medicine as a comprehensive interdisciplinary concept, which combines all the new methodological opportunities of genome research with its use in experimental research in the laboratory, the transfer of these results to the clinics and everyday general practice, addressing new questions in bioethics as well as the practical and commercial use of the results for the diagnosis, prevention and treatment of diseases affecting the cardiovascular and nervous systems as well as cancer.

Over the last ten years we have established a research system to achieve exactly this on the Berlin-Buch Campus. In addition to the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch and the basic research of the Forschungsinstitut für Molekulare Pharmakologie, there are clinical applications at the Robert Rössle Cancer Center (German abbrev. RRK) and the Franz Volhard Clinic for Cardiovascular Diseases (German abbrev. FVK) and the Buch Clinic as well as the commercial applications of our research results in the Biotechnology Park with its 40 companies. This system also includes university teaching. The Life Science Learning Laboratory and the MDC.C, the clinics and the MDC take actively part in the debate about the future of medicine and the role of bioethics. In this context, Berlin-Buch can be seen as a suitable model for a future knowledge-based world. Science will shape our future.

Detlev Ganten

riskiert hätte, solche Prognosen abzugeben. Jetzt kommen sie von erfolgreichen Wissenschaftlern und machen Sinn. Man kann sie nachvollziehen und dank der Molekularen Medizin für realistisch halten. Auf dem Campus in Berlin-Buch arbeiten wir mit daran, sie in die Tat umzusetzen. Die nächsten zehn Jahre werden zeigen, wie weit wir dabei kommen. Sicher ist, dass die ärztliche Fürsorge und menschliche Zuwendung in Zukunft eher noch wichtiger wird.

Mit dem Begriff „Molekulare Medizin“ verbinden wir in Berlin-Buch mehr als den Einsatz von Methoden der Molekularbiologie, Gentechnologie und Genomforschung für das Verständnis der molekularen biologischen Mechanismen bei Gesundheit und Krankheit. Wir verstehen die Molekulare Medizin als ein umfassendes interdisziplinäres Konzept, das die neuen methodischen Möglichkeiten der Genomforschung in ihrer ganzen Breite mit der Anwendung in der experimentellen Forschung im Labor und der Übertragung der Ergebnisse in die Klinik und in die ärztliche Praxis verbindet. Dazu gehört weiter die praktische und wirtschaftliche Nutzung der Ergebnisse für Diagnose, Vorbeugung und Therapie von Krankheiten im Bereich Herz-Kreislauf, Krebs und des Nervensystems sowie die Auseinandersetzung mit neuen Fragen der Bioethik.

Wir haben auf dem Campus in Berlin-Buch dazu in den vergangenen 10 Jahren ein Forschungssystem aufgebaut. Dieses umfasst neben dem Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch und der Grundlagenforschung des Forschungsinstitutes für Molekulare Pharmakologie (FMP), die klinische Anwendung in der Robert-Rössle-Krebsklinik (RRK) und Franz-Volhard-Herz-Kreislauf-Klinik (FVK) und dem Klinikum Buch und die wirtschaftliche Nutzung der Ergebnisse im Biotechnologiepark mit seinen 40 Firmen aber auch die Lehre an den Universitäten. Mit dem Gläsernen Labor und dem MDC.C sowie in verschiedenen nationalen und internationalen Gremien beteiligen wir uns aktiv an der Debatte über die Fragen zur Zukunft der Medizin und zur Rolle der Bioethik. Berlin-Buch kann auf diese Weise zu einer erlebbaren Modellregion einer zukünftigen humanen Wissensgesellschaft werden. Die Wissenschaft wird unsere Zukunft prägen.

Detlev Ganten

Overview

Überblick

Clinical Research

The collaboration between the MDC and the two university clinics, the Franz Volhard Clinic for Cardiovascular Diseases (FVK) and Robert Rössle Cancer Center (RRK), have developed in an exceedingly satisfactory manner in recent years. Annually, the MDC makes available about 7 million Euro from its budget for joint research projects. The following procedures have been set up to allocate MDC research funds to clinics:

The four heads of departments of the FVK and RRK also head research groups at the MDC. This means that the clinicians are part of the MDC research activities and members with equal rights on all committees of the MDC, including the management working party. This ensures that there is an unhindered institutional link between clinical and basic research. The same refers to teaching activities at the universities.

Clinical Research Units (CRUs) form the basis of collaborative projects. These CRUs are not involved in routine patient care but are exclusively intended for diagnostic procedures and the scientific investigation of patients required as part of the collaborative projects. In this respect, they represent an unusual feature of the research structure of a university clinic. Examples of MDC-funded measures include the equipping of a center for the identification of genetic diseases and for establishing experimental protocols in the field of circulatory diseases at the FVK as well as setting up a tumor bank at the RRK. This is available to all researchers for their research projects and provides a useful service involving future high-throughput procedures based on DNA chip technology to help identify altered disease genes.

All the research funding which the MDC provides for Collaborative Projects (“Twinning Grants”) between clinicians and basic research groups is monitored by internal and external experts.

Klinische Forschung

Die Zusammenarbeit zwischen dem MDC und den beiden Universitätskliniken – der Franz-Volhard-Klinik für Herz-Kreislauferkrankungen (FVK) und der Robert-Rössle-Krebsklinik (RRK) hat sich in den vergangenen Jahren sehr gut entwickelt. Das MDC stellt jährlich etwa sieben Millionen Euro aus seinem Budget für gemeinsame Forschungsvorhaben zur Verfügung. Für die Vergabe von MDC-Forschungsgeldern in die Kliniken wurden folgende Verfahren eingerichtet:

Die vier leitenden Ärzte der FVK und der RRK leiten gleichzeitig eine eigene Forschungsgruppe am MDC. Als MDC-Forschungsgruppenleiter sind sie zugleich Mitglieder in allen MDC-Gremien einschließlich des Leitungskollegiums. Damit wird eine institutionelle Verbindung zwischen Klinik und Grundlagenforschung sichergestellt.

Klinische Forschungsgruppen bilden die Basis der gemeinsamen Projekte. Sie beteiligen sich nicht an der Routineversorgung der Patienten, sondern ausschließlich an diagnostischen Verfahren und wissenschaftlichen Untersuchungen von Patienten im Rahmen von gemeinsamen Forschungsprojekten. Sie stellen in dieser Hinsicht eine Besonderheit in der Forschungsstruktur einer Universitätsklinik dar.

Zu den Beispielen der vom MDC geförderten Maßnahmen gehören die Ausstattung eines Zentrums für die Identifizierung genetisch bedingter Krankheiten sowie die Etablierung experimenteller Protokolle auf dem Gebiet der Herz-Kreislauferkrankungen an der FVK oder einer Tumorbank an der RRK. Diese Einrichtungen stehen allen Forschern für ihre Arbeiten zur Verfügung. Sie sind ein nützliches Instrument auch für die künftig verfügbaren Verfahren mit hohen Durchlaufkapazitäten, die auf der Chip-Technologie basieren und dabei helfen, veränderte Gene, die eine Bedeutung für die Entstehung von Krankheiten haben könnten, zu identifizieren.

Die gesamte Forschungsförderung, die das MDC für die gemeinsamen Projekte („Twinning Grants“) von Klinikern und

The multitalented molecules of molecular medicine

NF-κB: a protein that switches on genes

NF-κB is the name for a small group of related proteins that are to be found in all cells in the body. They normally exist in their inactive form in the cytosol but, after the external effect of a variety of signaling agents, like cytokines, growth factors or bacterial pathogens, the NF-κBs are transported from the cytosol into the cell nucleus where they bind to the genomic DNA. This triggers the copying of a group of genes which results in a subsequent increase in the proteins coded by these genes. These proteins, which are under the control of NF-κB, include a number of important switching molecules, some of which control cellular growth properties and programmed cell death, while others can trigger signal-regulated gene activation in other cells. A number of studies have shown that NF-κB- regulated signal-dependent gene regulation is a pre-requisite for the correct functioning of the cells of the immune system. No humoral or cellular immune reactions can take place without NF-κB. Since NF-κB activates the expression of certain cytokines, such as tumor necrosis factor and a number of interleukins, it also plays a key role in chronic inflammation. NF-κB-associated conditions include a wide spectrum of autoimmune and inflammatory conditions, such as rheumatoid arthritis, psoriasis and other skin conditions, asthma, Crohn's disease and atherosclerosis. Due to its anti-apoptotic and cell-division-activating effects, NF-κB is of general importance as a potential therapeutic target as far as the resistance of tumors to chemo- or radiotherapy is concerned. Defective cellular control of NF-κB is considered to be a critical step in the pathogenesis of certain lymphomas (e.g. Hodgkin's disease) and leukemia. Biochemical and biological investigations of NF-κB have provided fundamental information which is making an increasing impact on clinical research. Due to its widespread clinical significance, MDC researchers have been studying NF-κB for a long time at both a basic level and in collaboration with its two clinics.

For further details see the report of *Claus Scheidereit*'s research group

Die vielseitigen Moleküle der Molekularen Medizin

NF-κB: ein Eiweiß, das Gene anschaltet

NF-κB bezeichnet eine kleine Gruppe verwandter Proteine, die in allen Zelltypen des Körpers vorkommen. Dort befinden sie sich in inaktiver Form im Zytosol. Nach äußerer Einwirkung verschiedener als Signale wirkender Substanzen, wie Zytokine, Wachstumsfaktoren oder bakterieller Pathogene, werden die NF-κBs aus dem Zytosol in den Zellkern transportiert und binden dort an die genomische DNA. Hierdurch wird die Abschrift einer Gruppe von Genen aktiviert, was zu einer nachfolgenden Zunahme der von diesen Genen kodierten Proteine führt. Diese durch NF-κB kontrollierten Proteine umfassen eine Anzahl von wichtigen Schaltermolekülen, von denen einige das zelluläre Wachstumsverhalten und den programmierten Zelltod steuern, andere wiederum eine Signal-vermittelte Genaktivierung in anderen Zellen auslösen können. Eine Anzahl von Untersuchungen haben gezeigt, daß NF-κB vermittelte Signal-abhängige Genregulation eine Voraussetzung für die Funktionsweise von Zellen des Immunsystems ist. Weder humorale noch zelluläre Immunreaktionen können ohne NF-κB zustande kommen. Da NF-κB die Expression von bestimmten Zytokinen, wie Tumornekrosisfaktor und eine Reihe von Interleukinen aktiviert, spielt es auch eine Schlüsselrolle bei chronischen Entzündungen. NF-κB-assoziierte Erkrankungen schließen ein weites Spektrum von Autoimmun- und Entzündungserkrankungen ein, wie rheumatoide Arthritis, Psoriasis und andere Dermatosen, Asthma, Morbus Crohn und Artherosclerose. Wegen seiner anti-apoptotischen sowie zellteilungs-aktivierenden Wirkungen erlangt NF-κB bei der Resistenz von Tumoren gegen Chemo- oder Radiotherapie als mögliches therapeutisches Target eine generelle Bedeutung. Eine defekte zelluläre Kontrolle von NF-κB wird zudem bei bestimmten Lymphomen (z. B. Morbus Hodgkin) und Leukämien als einer der entscheidenden Schritte der Krankheitsentstehung angenommen.

Biochemische und biologische Untersuchungen von NF-κB haben ein umfassendes Grundlagenwissen geschaffen, welches in zunehmendem Maße auf die klinische Forschung Einfluß nimmt. Wegen der breiten klinischen Signifikanz werden am MDC seit längerem NF-κB Forschungsarbeiten nicht nur im Grundlagenlabor, sondern auch in Kooperation mit den beiden Kliniken durchgeführt.

Weitere Einzelheiten im Bericht der Arbeitsgruppe von *Claus Scheidereit*

The clinical research supported by MDC funds is based exclusively on research projects. These projects obtain financial support for research-associated additional expenditure provided that it involves collaborative projects between the MDC and the clinics. These projects are proposed following close agreement with the coordination sectors of the MDC and, as far as the topics are concerned, they are classified under the research specialities of molecular therapy, genetics, cell growth and differentiation as well as neurosciences. In general, such projects run for 2–3 years. The maximum period of funding is 5 years.

This allows the application of research methods under quality-controlled conditions and the support of collaboration between basic research and the clinics. In addition, the two clinics have the opportunity to gain access to third-party funding, independently of the MDC, to help support their clinical research.

The fundamental idea behind a special clinical training program for young doctors is to use targeted trainee sponsorship of doctors who have completed their clinical training to facilitate the switch to basic research at the MDC and to lay the foundation for their further scientific development. At present 16 young physicians are taking part in this training program. These four MDC-sponsored programs for clinical research

Gruppen der Grundlagenforschung aufbietet, wird von internen und externen Experten überwacht.

Die Finanzierung der klinischen Forschung aus MDC-Mitteln erfolgt ausschließlich für gemeinsame Forschungsprojekte mit dem MDC. Diese Projekte werden in enger Übereinstimmung mit dem MDC vorgeschlagen und thematisch den Forschungsschwerpunkten des MDC zugeordnet, also der Molekularen Therapie, Genetik, Zellwachstum und Zelldifferenzierung und den Neurowissenschaften. Im allgemeinen haben diese Projekte eine Laufzeit von zwei bis drei Jahren. Die maximale Förderdauer liegt bei fünf Jahren.

Damit wird die Unterstützung der Zusammenarbeit zwischen Grundlagenforschung und klinischer Forschung sowie der Einsatz von Forschungsmethoden und ihrer Qualitätskontrolle sichergestellt. Daneben haben die beiden Kliniken die Möglichkeit, sich Zugang zur Drittmittelförderung – unabhängig vom MDC – zu verschaffen.

Grundidee eines speziellen Ausbildungsprogramms für junge Ärzte ist es, Medizinern mit abgeschlossener klinischer Ausbildung gezielt den Übergang in die Grundlagenforschung am MDC zu erleichtern und die Basis für ihre weitere wissenschaftliche Entwicklung zu legen. Zur Zeit nehmen 16 junge Ärzte an diesem Ausbildungsprogramm teil.

form a unit and have been set up by agreed procedures to guarantee that outstanding clinical research projects can be supported for specific periods from MDC resources in the RRK and FVK.

To bring together the two different worlds of basic and clinical research was quite unique in Germany in 1992. However, this model of close collaboration between these two worlds has turned out to be very promising.

One example of this is the area of molecular therapy. The MDC and the clinics have developed various strategies for gene therapeutic approaches. The MDC and the clinics have come to an agreement with other working groups and institutions in conjunction with the Union of Clinical Pharmacology Berlin/Brandenburg and obtained substantial funding from the BMBF for a period of 8 years.

Moreover, laboratories meeting the good manufacturing practice standards (GMP laboratories) have been set up in the MDC in conjunction with one partner of the pharmaceutical industry, Schering AG (Berlin). This GMP facility involves four laboratories for vector production and two for analysis and cell culture. It is also available for use in collaborative projects with other partners e.g. biotechnology companies located on the Campus.

Diese vier Programme für die klinische Forschung bilden eine Einheit. Sie sind nach gemeinsam aufgestellten Kriterien eingerichtet und begutachtet worden. Damit wird sichergestellt, dass hervorragende klinische Forschungsprojekte in der RRK und FVK zeitlich befristet mit MDC-Mitteln gefördert werden können.

Es war im Deutschland des Jahres 1992 einzigartig, die beiden so verschiedenen Welten von Grundlagenforschung und klinischer Forschung zusammenzubringen. Das Modell der engen Zusammenarbeit zwischen diesen beiden Bereichen hat sich jedoch viel versprechend entwickelt.

Ein Beispiel betrifft dabei das Gebiet der Molekularen Therapie. Das MDC und die Kliniken haben verschiedene gentherapeutische Strategien entwickelt. Das MDC und die Kliniken konnten sich zu diesen Arbeiten mit anderen Gruppen und Institutionen abstimmen und zusammen mit dem Verbund für Klinische Pharmakologie Berlin/Brandenburg erhebliche Fördermittel des Bundes (BMBF) für den Zeitraum von acht Jahren einwerben.

Darüber hinaus konnte das MDC mit einem Partner aus der pharmazeutischen Industrie – der Schering AG –, GMP-Laboratorien (GMP – Good Manufacturing Practice) einrichten. Diese GMP Einrichtung verfügt über vier Labore zur Vektorproduktion und zwei für Analyse und Zellkultur. Die Laboratorien stehen für Projekte mit anderen Partnern – wie etwa Biotechnologiefirmen auf dem Campus – zur Verfügung.

GTB GenTherapeutika Berlin-Buch GmbH

GTB GenTherapeutika Berlin-Buch is a company specializing in the manufacture of clinical trial samples for clinical studies in phases I, II and III. The trial samples are viral and non-viral vectors as well as liposomal carrier systems for gene therapy, cellular products and oncolytic viruses. It was founded in August 2000 and is a cooperation between the pharmaceutical company Schering AG Berlin and the Max Delbrück Center for Molecular Medicine (MDC) in Berlin-Buch.

GTB GenTherapeutika is a contract development and manufacturing organization. Besides manufacturing clinical trial samples, the company also offers manufacture of non-GMP material for pre-clinical studies and development of lab scale processes for GMP-compatible processes. As manufacturer of biopharmaceutical products, GTB GenTherapeutika has established a quality management and a quality assurance system according to German laws and regulations governing the manufacture of drugs.

The manufacture facility has been established as a cleanroom facility. The facility consists of several cleanrooms which represent the cleanroom classification A, B and D. Cleanroom areas of categories A and B are equipped and devised for aseptic works, the areas of category D are for analytics (molecular-biological and cell-based analytics) and for product storage (cryo cell storage). The entire facility as well as the single processing steps are being monitored by a complex supervision and documentation system. The cleanroom facility and the operational equipment of the clean room areas are qualified, validated where necessary and in compliance with the current GMP guidelines of the EU and the FDA (USA). In addition, the facility is suitable for work with genetically modified organisms of Risk Group 2 (GenTG).

GTB GenTherapeutika Berlin-Buch GmbH

Die GTB GenTherapeutika Berlin-Buch GmbH ist ein Unternehmen, das sich auf die Herstellung von Proben für klinische Versuche spezialisiert hat, die für Arzneimittelstudien in den klinischen Phasen I, II und III eingesetzt werden. Die Versuchsproben enthalten neben viralen und nicht-viralen Vektoren auch liposomale Transportsysteme für die Gentherapie und darüber hinaus zelluläre Produkte und onkolytische Viren. Das Unternehmen ist im August 2000 gegründet worden, und zwar als Kooperation zwischen der Schering AG und dem MDC.

Die GTB GenTherapeutika Berlin-Buch GmbH agiert als Organisation im Bereich der Kontraktentwicklung und der Herstellung. Neben der Anfertigung von klinischen Versuchsproben bietet das Unternehmen die Herstellung von Materialien für präklinische Studien an, die nicht den GMP Richtlinien unterliegen, und es entwickelt Abläufe im Labormaßstab für solche Verfahren, für die der GMP Standard verlangt wird. Als Hersteller von biopharmazeutischen Produkten hat die GTB GenTherapeutika Berlin-Buch GmbH ein Qualitätsmanagement und ein Qualitätssicherungssystem entwickelt, das den deutschen Arzneimittelgesetzen Rechnung trägt.

Die Produktionsanlagen sind als „cleanroom facilities“ (Reinräume) angelegt. Sie bestehen aus mehreren Reinräumen entsprechend der Reinraum-Klassifikation A, B und D. Zonen der Kategorie A und B sind ausgerüstet für aseptische Arbeiten, während die Kategorie D für analytische Arbeiten (molekularbiologischer und zellanalytischer Art) und Produktlagerung (eingefrorene Zellen) vorgesehen ist. Sowohl die gesamte Anlage als auch die einzelnen Verarbeitungsschritte unterliegen einem komplexen Überwachungs- und Dokumentationssystem. Die Reinraum-Einrichtungen und die operationelle Ausrüstung sind qualifiziert, wenn nötig validiert und in Übereinstimmung mit den derzeitigen GMP Richtlinien der EU und der amerikanischen FDA. Daneben ist die Anlage auch für genetisch modifizierte Organismen der Risikogruppe 2 (GenTG) geeignet.

A new partner: the Helios-Kliniken GmbH

Since June 2001, the Franz Volhard Clinic for Cardiovascular Diseases and Robert Rössle Cancer Clinic of the "Charité" Medical School of the Humboldt University of Berlin, have privatized their health care facilities which are now managed by Helios-Kliniken GmbH. This will ensure that, in the future, healthcare and research will be provided by means of close cooperation between the Humboldt University and the private sector. Helios-Kliniken GmbH will erect a new clinic costing approximately 200 million Euro in Berlin-Buch and incorporate the Berlin-Buch university clinics of the Charité, FVK and RRK, in this new building together with the former 1000 bed Community Hospital. Thus, one of the most modern and largest clinics of Berlin will be located in the vicinity of the Berlin Buch campus.

Plans for a new Medical Genomics Building at the MDC

The Max Delbrück Center is planning to set up a new research facility on the Berlin-Buch campus, a laboratory building for Medical Genomics. It will be devoted to the application of modern genomics for the analysis of gene functions in health and complex genetic diseases. The MDC staff has developed a concept which has been refined in expert meetings with scientists from various European countries and the USA. The concept has also been presented to the Federal Ministry of Education and Research and the Senate of Berlin. The Scientific Advisory Board of the MDC approved the concept in July 2000.

The following research technologies were identified as of primary importance for future research in the center for medical genomics: (i) bioinformatics, (ii) structural biology, (iii) human genetics and epidemiology, (iv) genetics of animal models and (v) the genome-wide analysis of the expression of genetic information. These technologies are to be aimed at the analysis of normal physiological processes and complex dis-

Bundesgesundheitsministerin Ulla Schmidt (Mitte) mit MDC-Stiftungsvorstand Prof. Detlev Ganten (2.v.re) sowie Klinikern und Wissenschaftlern bei ihrem Besuch des Campus Berlin-Buch am 8. Mai 2001.
Federal Secretary of Health, Ulla Schmidt (in the middle), listening to MDC's Scientific Director Detlev Ganten (2nd from right) during her visit to the Berlin-Buch Campus on May 8, 2001, as clinicians and researchers from Buch look on.
Copyright: MDC/Uwe Eising

Ein neuer Partner – die Helios-Kliniken GmbH

Seit Juni 2001 ist die Patientenversorgung der FVK und der RKK (beide gehören zur Charité, der Medizinischen Fakultät der Berliner Humboldt-Universität) privatisiert und wird von der Helios-Kliniken GmbH gemanagt. Zukünftig ist damit sicher gestellt, dass Krankenversorgung und Forschung in enger Kooperation von Humboldt-Universität und dem privaten Träger erfolgen können. Helios-Kliniken GmbH wird in Berlin-Buch eine neue Klinik für rund 200 Millionen Euro errichten und die Bucher Universitätskliniken der Charité, der FVK und des RRK, zusammen mit dem Bucher Klinikum mit ehemals 1.000 Betten, in das neue Gebäude einbeziehen. Damit wird sich eine der modernsten und größten Kliniken von Berlin in der Nähe des Buches Campus befinden.

Der frühere Wissenschaftssenator Dr. Christoph Stölzl (2. v. l.) besuchte am 3. Juli 2000 das MDC. Mit dabei (v.l): Prof. Bernd Dörken (damaliger Ärztlicher Leiter der Robert-Rössle-Klinik der Charité), Dr. Gudrun Erzgräber (Geschäftsführerin der BBB Management GmbH Campus Berlin-Buch), Prof. Rainer Dietz (Ärztlicher Leiter der Franz-Volhard-Herz-Kreislauf-Klinik) und Prof. Detlev Ganten (MDC-Stiftungsvorstand).
Former Berlin Senator for Science, Dr. Christoph Stölzl (2nd from left) visited the MDC on July 3rd, 2000. With him (from left) are: Prof. Bernd Dörken (former Medical Director of the Robert Rössle Cancer Center of the Charité), Dr. Gudrun Erzgräber (Managing Director of the BBB Management GmbH Campus Berlin-Buch), Prof. Rainer Dietz (Medical Director of the Franz Volhard Clinic for Cardiovascular Diseases of the Charité) and Detlev Ganten (MD, Ph.D, MDC's Scientific Director).
Copyright: MDC/Uwe Eising

Pläne für ein neues Gebäude für Medizinische Genomforschung am MDC

Das MDC plant, eine neue Forschungseinrichtung auf dem Campus Berlin-Buch zu bauen, und zwar ein Laborgebäude für Medizinische Genomforschung. Es wird sich der Anwendung der modernen Genomforschung durch die Analyse von Genfunktionen für die Gesundheit und für komplexe genetische Krankheiten widmen. Am MDC ist dafür ein Konzept entwickelt worden, das in Fachtagungen mit Wissenschaftlern aus verschiedenen europäischen Ländern und den USA weiter ausgearbeitet worden ist. Das Konzept ist ebenfalls dem Bundesministerium für Bildung und Forschung und dem

eases (neurological diseases, cancer, cardiovascular or metabolic diseases). A major financial contribution is to be provided by EFRE (European Fund for Regional Development). The architects have been chosen, and the laboratory building is planned to be ready for use in September 2004.

Biotechnology Park with its Innovation and Start-up Center

Since the beginning of the 1990s, right next to the basic research and clinical activities, there has been a technology park on the Berlin-Buch Campus with an Innovation and Start-up Center (German abbrev IGZ) where small and medium-sized companies can carry out research and production activities. At present, 39 companies are located on the campus. Of these, 31 are biotechnology companies and the remaining eight are involved in providing biotech support and services. The companies employ about 550 staff in all.
Young and start-up companies are currently located in five modern buildings on the IGZ site covering an area of about 14,100 square meters of attractive specially adapted space. In 2001, BBB Management GmbH Campus Berlin-Buch, the company responsible for the management and development of the campus, took over the running of another two buildings, the Otto Warburg House (House 80) and the Karl Lohmann House (House 82). With their laboratory and office space, these buildings are able to meet the needs of a series of companies including three newcomers (Combinature Biopharm AG, Custos Biotech AG and Semaia GmbH).

Die früheren Berliner Senatoren Wolfgang Branoner (re.) und Dr. Christoph Stölzl am 13. Juli 2000 bei der Grundsteinlegung für das MDC-Kommunikationszentrums.
Two former Berlin Senators, Wolfgang Branoner (right) and Dr. Christoph Stölzl laying the foundation stone of the Max Delbrück Communications Center (MDC.C).
Copyright: BBB GmbH

In September 2001, building was started on the third stage of the IGZ. BBB Management GmbH is providing support in the region of 21 million Euro. The extension with its 8,500 m² working area will be available to future users by the first quarter of 2003. The building is designed so that future users, especially those engaged in bioinformatics, will have a suitable place to carry out their activities on the campus. Completion of this third and final stage of its building program means

Senat von Berlin vorgestellt worden. Der Wissenschaftliche Beirat des MDC hat dem Konzept im Juli 2000 zugestimmt.

Die folgenden Forschungstechniken wurden als äußerst wichtig für künftige Forschungen im Zentrum für Medizinische Genomforschung angesehen. (1) Bioinformatik und (2) Strukturbiologie, (3) Humangenetik und Epidemiologie, (4) Genetik von Tiermodellen und (5) Genomweite Analyse der Genexpression. Diese Technologien sollen zur Analyse gesunder physiologischer Prozesse und von komplexen Krankheiten (neurologischen Erkrankungen, Krebs, Herz-Kreislauf- oder Stoffwechselstörungen) genutzt werden. Ein maßgeblicher finanzieller Beitrag wird vom Europäischen Fonds für die Regionalförderung (EFRE) erwartet. Die Architekten sind ausgewählt, und das Laborgebäude könnte im September 2004 bezugsfähig sein.

Biotechnologiepark mit Innovations- und Gründerzentrum

Im direkten Umfeld von Grundlagenforschung und klinischer Praxis ist auf dem Campus Berlin-Buch seit Beginn der 1990er Jahre ein Technologiepark mit Innovations- und Gründerzentrum (IGZ) entstanden, in dem kleine und mittelständige Unternehmen forschen und produzieren. Gegenwärtig sind auf dem Campus 39 Unternehmen angesiedelt. Davon sind 31 Biotechnologieunternehmen, die verbleibenden acht Unternehmen sind in den Bereichen Support und Services für Biotechs tätig. Die Firmen beschäftigen insgesamt circa 550 Mitarbeiter.

Junge Unternehmen und Existenzgründer finden im IGZ derzeit in fünf modernen Gebäuden auf 14.100 Quadratmetern branchenspezifische, attraktive Rahmenbedingungen. 2001 nahm die BBB Management GmbH Campus Berlin-Buch, die Betreiber- und Entwicklungsgesellschaft des Campus, mit dem Otto-Warburg-Haus (Haus 80) und dem Karl-Lohmann-Haus (Haus 82) zwei weitere Gebäude in Betrieb. Mit diesen beiden Labor- und Bürogebäuden konnte zunächst der Bedarf einer Reihe von Unternehmen nach Erweiterung sowie von drei Neugründungen (Combinature Biopharm AG, Custos Biotech AG und Semaia GmbH) nach Ansiedlung auf dem Campus befriedigt werden.

Im September 2001 wurde mit der Errichtung der 3. Ausbaustufe des IGZ begonnen. Der BBB Management GmbH stehen dafür Fördermittel in Höhe von insgesamt etwa 21 Millionen Euro zur Verfügung. Der Erweiterungsbau mit 8.500 m² Nutzfläche wird bis zum I. Quartal 2003 den künftigen Nutzern zur Verfügung stehen. Das Gebäude ist so ausgelegt, dass künftig insbesondere auch Bioinformatik-Unternehmen auf dem Campus angesiedelt werden können. Mit dieser dritten Baustufe wird das IGZ mit insgesamt ca. 22.500 m² seine Endausbaustufe erreichen. Damit verfügt der Campus über eines der größten branchenspezifischen Gründerzentren in Deutschland.

Die BBB Management GmbH verfolgt zielstrebig Pläne zur Erweiterung des Biotechnologieparks über den jetzigen Campus hinaus. Im Mittelpunkt stehen Firmenentwicklungen außerhalb des IGZ-Bereichs mit Flächenbedarf um oder über

Dr. Gudrun Erzgräber (l.) von der BBB Management GmbH Campus Berlin-Buch und die Berliner Bildhauerin Sabina Grzimek enthüllen die Büste von Otto Warburg bei der Eröffnung des Otto-Warburg-Hauses am 12. Oktober 2001.
Dr. Gudrun Erzgräber (left), BBB Management GmbH Campus Berlin-Buch, and the Berlin sculptress, Sabina Grzimek, unveil the bust of Otto Warburg at the opening of the Otto Warburg House on October 12, 2001.
Copyright: BBB GmbH; Stefan Kühl, Helios Klinikum Berlin

that the IGZ will cover an area of ca. 22,500 m². This will provide the campus with one of the largest specialized start-up centers in Germany.

BBB Management GmbH is determined to pursue its plans for the expansion of the Biotechnology Park beyond the limits of the present campus. At the heart of this are business developments over and above that of the IGZ requiring 2,000 m² or more of space. BBB Management GmbH is working hard to convert the site occupied by the former Section V of the Clinic (Hobrechtsfelder Chaussee Campus). According to the renovation plans of the BBB, after renovation and modernization, there will be a 15 hectare facility which will provide ca. 14,000 m² office and laboratory space and this will be followed by new building work to provide a further 14,000 m² of working space.

Due to the activities of BBB GmbH, the campus has taken on the character of a biomedical research and business park. The close collaboration between research, the clinics and the companies makes this a very lively campus.

BBB GmbH manages the campus's modern broad-band communications system, and is an active partner in Berlin, national and international networks and is responsible for targeted marketing for the Campus.

Since it was set up in 1995, BBB GmbH has invested about 65.3 million Euro in the expansion and modernization of the campus and its Biotechnology Park. This involves funding from the joint task force for the improvement of regional economic structures (German abbrev GA) and the European Regional Development Fund.

2.000 m². Die BBB Management GmbH arbeitet intensiv an der Nachnutzung des ehemaligen örtlichen Bereiches V des Klinikums (Campus Hobrechtsfelder Chaussee). Das Nachnutzungskonzept der BBB sieht für das 15 ha große Areal vor, in Bestandsgebäuden durch Sanierung und Modernisierung ca. 14.000 m² Büro- und Laborfläche und sukzessive weitere Neubauten mit nochmals insgesamt 14.000 m² Nutzfläche zu schaffen.

Durch die Tätigkeit der BBB GmbH hat der Campus den Charakter eines biomedizinischen Wissenschafts- und Wirtschaftsparks erhalten. Das enge Zusammenwirken von Forschung, Kliniken und Unternehmen prägt das Campusleben. Die BBB GmbH betreibt das moderne Breitbandkommunikationssystem des Campus, ist aktiver Partner in Berliner, nationalen und internationalen Netzwerken und führt das Standortmarketing für den Campus durch. Seit ihrer Gründung 1995 hat die BBB GmbH rund 65,3 Millionen Euro in den Ausbau und die Modernisierung des Campus sowie seinen Biotechnologiepark investiert. Darin sind Mittel der Gemeinschaftsaufgabe zur Verbesserung der regionalen Wirtschaftsstruktur (GA) und des Europäischen Fonds für Regionale Entwicklung enthalten.

Die BBB GmbH wurde durch das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch gegründet. Mitgesellschafter sind das Forschungsinstitut für Molekulare Pharmakologie (20 %) sowie die Schering AG (20 %).

Das ehemalige Dr. Heim-Krankenhaus in Berlin-Buch – Standort für die Erweiterung des Biotechnologieparks
The former Dr. Heim Hospital in Berlin-Buch – site for expansion of the Biotechnology Park
Copyright: BBB GmbH/Annett Krause

BBB GmbH was set up by the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch. Its other partners are the Research Institute for Molecular Pharmacology (20%) and Schering AG (20%).

Life Science Learning Laboratory and activities involving the general public

The activities involving the general public organized by the Campus Users Committee has been able to handle the growing interest in the Campus by politicians, management, professional bodies from business and industry as well as the public in the from of visits by delegations, on-site meetings, campus and company visits, inquiries and events. The Life Science Learning Lab, headed by Dr. Ulrich Scheller, has fulfilled the increasing need for a centrally located Info-Center for the Campus. Since 1999, the Life Science Learning Lab has offered practical instruction in biology for children from highschools. Annually, about 3,500 pupils mainly from Berlin and Brandenburg visit the Life Science Learning Lab. In addition to school courses, the Life Science Learning Lab also plays an important role in teacher training and the development of new pupil experiments – which ideally should be able to be carried out in every school. A 5-day intensive course for pupils is offered during the vacation period to encourage pupils interested in the natural sciences.

Since September 2001, the Life Science Learning Lab has been successfully associated with the Berlin model project "Life Science in Schools". The aim of this project supported by the regional schools office, BIOTOP and the TSB is to allow over 5000 pupils a year to gain practical experience of working in a laboratory. Under the overall control of the Life Science Learning Lab, similar courses have also been held since Autumn 2001 at the Professional Education Chemistry Center in Adlershof as well as the "Lise Meitner" and "Emil Fischer" upper-school centers.

Besucher experimentieren im Gläsernen Labor bei der „Langen Nacht der Wissenschaften" am 15. September 2001
Visitors carry out experiments in the Life Science Learning Laboratory during the „Long Night of the Sciences" on September 15, September 2001
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Gläsernes Labor und Öffentlichkeitsarbeit

Die von der Nutzergemeinschaft des Campus gemeinsam getragene Öffentlichkeitsarbeit war in der Lage, das wachsende Interesse am Campus durch Politik, Verwaltung, Fachkreise aus Wirtschaft und Unternehmertum sowie Öffentlichkeit in Form von Delegationsbesuchen, Vorortterminen, Campus- und Firmenbesichtigungen, Anfragen und Veranstaltungen zu bewältigen. Das Gläserne Labor (Life Science Learning Lab) unter Leitung von Dr. Ulrich Scheller erfüllt zunehmend Aufgaben eines zentralen Info-Center für den Campus. Es bietet seit 1999 praktischen Biologieuntericht für Schüler der gymnasialen Oberstufe an. Pro Jahr besuchen rund 3.500 Schüler vorwiegend aus Berlin und Brandenburg die Kurse im Gläsernen Labor. Über die Schülerkurse hinaus liegt ein Schwerpunkt der Arbeit des Gläsernen Labors in der Weiterbildung von Lehrern, der Entwicklung von neuen Schülerexperimenten – die idealer Weise auch in jeder Schule realisierbar sein sollten. Für die Förderung des naturwissenschaftlichen Nachwuchs wird ein 5-tägiger Schülerintensivkursen in den Ferien angeboten.

Seit September 2001 ist das Gläserne Labor erfolgreich in das Berliner Modellprojekt „Life Science in die Schule" eingebunden. Ziel dieses vom Landesschulamt, BIOTOP und der TSB geförderten Projektes ist es, über 5.000 Schülern pro Jahr das praktische Erlebnis eines Labortags zu ermöglichen. Unter Federführung des Gläsernen Labors werden ähnliche Kurse seit Herbst 2001 auch am Berufbildungszentrum Chemie in Adlershof sowie an den Oberstufenzentren „Lise-Meitner" und „Emil-Fischer" angeboten.

Besucher in der Ausstellung des Gläsernen Labor während der „Langen Nacht der Wissenschaften" am 15. September 2001
Visitors to the Life Science Learning Laboratory's exhibition during the „Long Night of the Sciences" on September 15, 2001
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The Helmholtz Association

The MDC belongs to the Helmholtz Association of German Research Centres (Helmholtz-Gemeinschaft Deutscher Forschungszentren, HGF). The Helmholtz Association is Germany´s largest government-funded research organization consisting of 15 national research centers. A total of 10 Helmholtz Centers contribute to research in the health field. The major four Helmholtz Centers in health research are GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, DKFZ-Deutsches Krebsforschungszentrum Heidelberg, GBF-Gesellschaft für Biotechnologische Forschung mbH and the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch.

The members of the Helmholtz Association have a high research profile in various fields of medical research, particularly in the areas of basic, clinical and public health research and they have key skills in research fields with complex, interdisciplinary and long-term scientific programs.

Recently, the Helmholtz Association has been reorganized. Under the reforms, most of the Helmholtz Association´s annual budget will be earmarked for six research fields, among them health research. These activities and capacities are structured in programs which are of a long-term, but not permanent, nature. In future, the funding of the Helmholtz Centers will be program-oriented. This means that funding will take place through the provision of resources for programs aimed at long-term research objectives carried out on the basis of cooperation and competition with external evaluation. Program-oriented funding is intended to raise the collaboration between the legally autonomous Helmholtz Centers to an even higher standard.

The MDC takes part in three out of seven programs in the research field, Health. These are the programs Cancer Research, Cardiovascular and Metabolic Disease Research and the program Function and Dysfunction of the Nervous System. The strategic evaluation of health research of the Helmholtz Centers is scheduled for summer 2002. During the conduct of these programs, the four centres devoting more than 60 percent of their total budget to the research field, Health (DKFZ, MDC, GBF, GSF), will strive to harmonize their managerial structures. For example, by setting up a steering committee consisting of the managerial boards of the four centres in order to achieve very close coordination. Furthermore, it is being considered to establish one common scientific advisory board for future program evaluations.

Die Helmholtz Gemeinschaft

Das MDC gehört zur Helmholtz Gemeinschaft Deutscher Forschungszentren (HGF). Die HGF ist Deutschlands größte staatlich finanzierte Forschungsorganisation, die aus 15 nationalen Zentren besteht. Insgesamt zehn Helmholtz Zentren arbeiten auf den Gebiet der Gesundheit. Die vier größten Helmholtz Zentren sind das GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, das DKFZ Krebsforschungszentrum Heidelberg, die GBF-Gesellschaft für Biotechnologische Forschung mbH und das MDC. Die Mitglieder der HGF weisen hohe Forschungsprofile in verschiedenen Bereichen der medizinischen Wissenschaft auf, insbesondere bei der Erforschung der biologischen Grundlagen, der klinischen Anwendung und der allgemeinen, die Gesundheit fördernden Maßnahmen („public health research“). Sie verfügen über Schlüsselkompetenzen in Forschungsgebieten mit komplexen, interdisziplinären und langfristigen wissenschaftlichen Programmen.

Vor kurzem ist die HGF restrukturiert worden. Als Konsequenz der Reform wird der größte Teil des jährlichen Budgets auf sechs Forschungsfelder verteilt werden, von denen eines die Gesundheit ist. Innerhalb dieser Forschungsfelder werden die Aktivitäten und Kapazitäten in Programmen strukturiert, die zwar langfristig, aber nicht auf Dauer angelegt sind. In Zukunft wird die Mittelvergabe bei der HGF programmorientiert sein. Dies bedeutet, daß die Förderung durch die Vergabe von Mitteln für Programme vor sich geht, die langfristige Forschungsziele haben, die auf der Basis von Kooperation und Kompetition mit externer Evaluierung durchgeführt werden.

Das MDC nimmt an drei von sieben Programmen auf dem Gebiet der Gesundheit teil. Dies sind die Programme Krebsforschung, Erforschung von Herz-Kreislauferkrankungen und Stoffwechselkrankheiten sowie das Programm Funktion und Fehlfunktion des Nervensystems. Die strategische Bewertung der Gesundheitsforschung ist für den Sommer 2002 vorgesehen. Im Verlauf der Fortschritte in diesen Programmen werden die vier Zentren (DKFZ, MDC, GBF, GSF), die mehr als 60% ihres Gesamtbudgets dem Thema Gesundheit widmen, sich bemühen, ihre Managementstrukturen zu harmonisieren. So lassen sich zum Beispiel Lenkungsausschüsse einrichten, zu denen das Management der vier Zentren gehört, um eine möglichst enge Kooperation zu erreichen. Darüber hinaus ist vorgesehen, einen gemeinsamen Wissenschaftsausschuss für die kommenden wissenschaftlichen Evaluierungen einzurichten.

External Evaluation

During the period November 1996 to April 1998, three external evaluations of its entire research activities were carried out at the MDC and, in early 2002, there was an evaluation of the clinical research programs in which basic MDC researchers closely collaborated with the clinicians at the Robert Rössle and Franz Volhard Clinics. A comprehensive strategic evaluation as part of the HGF Health program took place in summer 2002.

The evaluation were conducted in accordance with the same basic principle as that employed by special research areas of the German Research Society (DFG). The evaluation committee spent two days on-site to allow them the opportunity to get to know the researchers who gave a number of presentations. Following this site visit, the evaluation committee prepared a report which included a number of recommendations. Following these recommendations, the MDC has undertaken a series of financial and structural measures following detailed discussions by the Scientific Management Board. The results of these measures are regularly monitored using a check-list.

Externe Evaluierung

In der Zeit von November 1996 bis April 1998 ist am MDC in drei externen Begutachtungen die gesamte Forschung evaluiert worden. Im Frühjahr 2002 sind die klinischen Forschungsprogramme begutachtet worden, in denen Grundlagenwissenschaftler des MDC mit den Klinikern der Robert-Rössle-Klinik und der Franz-Volhard-Klinik eng zusammenarbeiten, Eine umfassende strategische Evaluation im Rahmen des Gesundheitsprogramms der HGF erfolgt im Sommer 2002.

Die bisherigen Begutachtungen verliefen nach dem gleichen Grundprinzip wie bei den Sonderforschungsbereichen der Deutschen Forschungsgemeinschaft (DFG). Die Gutachterkommissionen hatten auf der Grundlage ausführlicher Unterlagen vor Ort zwei Tage lang Gelegenheit, die Wissenschaftlerinnen und Wissenschaftler bei Vorträgen und Laborbesuchen kennenzulernen. Im Anschluss an die „site visit“ erstellten die Gutachterkommissionen einen Bericht, in dem Empfehlungen abgegeben wurden.

In addition, the MDC has carried out an evaluation of its management procedures. This involved all aspects of the structure, organization, quality and financial efficiency of the infrastructure and management of the MDC. In 1998, 1999 and 2000 there were also three rounds of evaluations involving Staff and Social Affairs, Purchasing and Materials as well as Finance and Accounting Procedures.

The Helmholtz Society centers were also evaluated by the Scientific Advisory Board as part of a system evaluation in 2000. On March 16 and 17, 2000, the MDC had a visit from the Scientific Advisory Board. In January 2001 the results of the evaluation by the Scientific Advisory Board were published. Based on this expert report, a fundamental reorganization of the entire Helmholtz Association has taken place.

Graduate student education – dean of graduate students

The support and structure of graduate student education at the MDC is given extreme importance. We have established a graduate program to accommodate highly qualified candidates and prepare them for careers in research science. The program provides training and research opportunities at the highest level within existing resources of the MDC. Ph.D./M.D. students participate in lectures and seminars held at the MDC and gain broad knowledge in biomedical sciences. A strong student-advisor relationship is essential for superior academic performance and is the basis for development of students into independent and creative researchers.

The approximately 120 MDC graduate students elect representatives who negotiate graduate student affairs with the institute and interact closely with the elected dean of graduate students. In addition, the student representatives organize scientific and social meetings for their fellow graduate students such as the traditional annual Student Symposium.

Helmholtz fellows

Helmholtz Fellowships are intended to allow promising young scientists an early opportunity to carry out their own independent research. The Helmholtz Fellowships have been established for young scientists who have already demonstrated that they are capable of carrying out high quality research on their own but who do not yet qualify for C3-grade posts or equivalent positions with their own facilities and personal freedom. The fellowships support the positions for up to a maximum of five years. The fellows are given their own equipment and the cost of this is borne by the research group. Generally, working space and personal equipment is supplied by the research group of the institution they join. It is expected that support is provided by the research group to allow the research fellow full independence. The aim is to allow the Helmholtz Fellows during the period of their award to set themselves up as independent researchers and compete on an equal footing. They are also expected to apply for third-party funding in their own right. Fellows are chosen following a positive assessment on the basis of suggestions from the research groups involved.

Das MDC hat aufgrund der Empfehlungen der Gutachter finanzielle und strukturelle Maßnahmen ergriffen, die im Wissenschaftlichen Ausschuss des Kuratoriums detailliert diskutiert worden sind. Die Ergebnisse der Maßnahmen werden anhand einer Checkliste regelmäßig überprüft.

Darüber hinaus hat das MDC eine Begutachtung der Verwaltung durchgeführt. Diese bezog sich auf alle Fragen der Gliederung, Organisation, Qualität und Wirtschaftlichkeit von Infrastruktur und Verwaltung des MDC. Sie fand 1998, 1999 und 2000 in drei Runden, jeweils für die Abteilungen Personal- und Sozialwesen, Einkauf und Materialwirtschaft sowie Finanz- und Rechnungswesen statt.

Alle Zentren der Helmholtz Gemeinschaft wurden im Rahmen einer Systemevaluation im Jahre 2000 durch den Wissenschaftsrat evaluiert. Am 16. und 17. März 2000 wurde das MDC durch den Wissenschaftsrat besucht. Im Januar 2001 wurden die Ergebnisse der Begutachtung durch den Wissenschaftsrat veröffentlicht. Auf der Basis dieser Begutachtung erfolgte die grundlegende Reorganisation der gesamten Helmholtz Gemeinschaft.

Ausbildung von Doktoranden – Der Vertrauensdozent

Der Förderung und Struktur der Ausbildung von Doktoranden wird im MDC besondere Aufmerksamkeit geschenkt. Es wurde ein Graduiertenprogramm eingerichtet, um hochqualifizierte Kandidaten auf ihre Karriere in der Forschung vorzubereiten. Das Programm sieht Trainings- und Forschungsmöglichkeiten auf höchstem Niveau im Rahmen der vorhandenen MDC Ressourcen vor. Doktoranden nehmen an Vorlesungen und Seminaren des MDC teil und erlangen dabei ein breites Wissen auf dem Feld der biomedizinischen Wissenschaften. Eine enge Verbindung zwischen Student und Lehrer ist wesentlich für eine herausragende akademische Qualifikation, und sie stellt die Grundlage dafür dar, dass aus den Doktoranden unabhängige und kreative Forscher werden.

Es gibt rund 120 Doktoranden am MDC. Sie wählen ihre Vertreter, welche die Belange der Doktoranden mit dem Institut verhandeln und die engen Kontakt mit dem gewählten Vertrauensdozent haben. Darüber hinaus organisieren die Studentenvertreter wissenschaftliche und soziale Zusammenkünfte für ihre Kommilitonen wie zum Beispiel das traditionelle jährliche Doktoranden-Symposium.

Helmholtz-Stipendiaten

Helmholtz-Stipendien sind vorgesehen, um die frühe Unabhängigkeit junger, erfolgversprechender Wissenschaftler zu ermöglichen. Die Helmholtz-Stipendien werden für junge Wissenschaftler eingerichtet, die bereits nachgewiesen haben, dass sie hervorragende eigenständige wissenschaftliche Arbeit leisten, die aber sich noch nicht für C3-Stellen oder andere äquivalente Positionen mit eigener Ausstattung und persönlicher Unabhängigkeit qualifizieren. Die Stipendien finanzieren die Stelle des Stipendiaten für einen Zeitraum bis zu maximal fünf Jahren. Sie werden mit eigenen Sachmitteln ausgestattet,

Scientific Journals

The MDC is the site of several editorial offices.

Journal of Molecular Medicine (JMM)

The Journal of Molecular Medicine (JMM) is published monthly by Springer Verlag Heidelberg since 1995. The focus is in molecular medicine, a field which applies the methods and knowledge of molecular biology and gene technology to medical research, therapy, and disease prevention. JMM's goal is to bring together basic science and clinical medicine in the field of molecular and gene technology research, which has become particularly important for the progress of medicine in all aspects. The journal's editor-in-chief is Detlev Ganten, Scientific Director of the MDC.

Neuroforum

Neuroforum is the Newsletter of the German Neuroscience Society (Neurowissenschaftliche Gesellschaft). The journal was founded in 1994, Neuroforum gives an overview of the activities in the field of neuroscience research in Germany. The journal publishes review articles covering all aspects of neuroscience research. Besides that, Neuroforum publishes articles on key persons in the history of the neurosciences, meeting reports, methodological aspects, book reviews, opinions, portraits of industrial research institutions, information on educational and research programs, and news from the German Neuroscience Society. The journal is published by Spektrum Akademischer Verlag, Heidelberg, Germany. The editor-in-chief is Helmut Kettenmann.

Glia

Glia, founded in 1988, provides a dedicated forum for a broad range of experimental topics in the field of glial research and is an indispensible medium for scientific exchanges among researchers interested in neuroglial research. Original articles, short communications, review articles and Special Issues on the physiology, anatomy, pharmacology, chemistry, and pathology of glia are published. The publisher is WILEY-LISS, New York, USA, editors-in-chief are Bruce Ransom and Helmut Kettenmann.

die sich an den Kosten für die Arbeitsrichtung orientieren. Die räumliche und personelle Ausstattung soll im Allgemeinen durch die aufnehmende Arbeitsgruppe der Institution gewährleistet werden. Es wird erwartet, dass von dieser Arbeitsgruppe Unterstützung geleistet wird und die Unabhängigkeit des Wissenschaftlers gefördert wird. Ziel ist es, dass die Helmholtz-Stipendiaten während der Zeit ihres Stipendiums in unabhängige Positionen hineinwachsen und sich darum bewerben. Für selbst eingeworbene Drittmittel wird eine selbstständige Verantwortlichkeit vorgesehen. Das Auswahlverfahren erfolgt über geeignete Begutachtung auf Vorschlag der aufnehmenden Arbeitsgruppe.

Wissenschaftliche Journale

Das MDC ist Sitz mehrerer wissenschaftlicher Redaktionen.

Journal of Molecular Medicine (JMM)

Das JMM wird seit 1995 monatlich vom Springer Verlag publiziert. Die Zeitschrift konzentriert sich auf das Gebiet der molekularen Medizin, in dem die Methoden und die Ergebnisse der Molekularbiologie und Gentechnologie für die medizinische Forschung, Therapie und Prävention genutzt werden. Das Ziel des JMM besteht darin, Grundlagenforschung und klinische Medizin zusammenzubringen, weil das Zusammengehen von Molekularbiologie und Gentechnologie eine besondere Bedeutung für alle Aspekte des Fortschritt in der Medizin hat. Der verantwortliche Herausgeber der Zeitschrift ist Detlev Ganten, der wissenschaftliche Vorstand des MDC.

Neuroforum

Neuroforum ist das Mitteilungsblatt der deutschen Neurowissenschaftlichen Gesellschaft. Die Zeitschrift ist 1994 gegründet worden. Neuroforum bietet einen Überblick über die Aktivitäten auf dem Gebiet der Neurowissenschaften in Deutschland. Die Zeitschrift publiziert Übersichtsartikel zu allen Aspekten der neurowissenschaftlichen Forschung. Darüber hinaus publiziert Neuroforum Aufsätze über Schlüsselpersonen in der Geschichte der Neurobiologie, bringt Berichte über Konferenzen, stellt methodologische Aspekte vor, bringt Buchrezensionen, Porträts von Industrieforschungsinstitute, informiert über Ausbildungs- und Forschungsprogramme und meldet die Neuigkeiten der deutschen Neurowissenschaftlichen Gesellschaft. Die Zeitschrift erscheint im Spektrum Akademischer Verlag, Heidelberg. Der Herausgeber ist Helmut Kettenmann.

Glia

Glia wurde 1988 gegründet und stellt ein engagiertes Forum für einen weiten Bereich an experimentellen Themen aus der Gliazellenforschung dar. Es ist ein maßgebliches Medium für den wissenschaftlichen Austausch unter Forschern in diesem Feld. Glia publiziert Originalbeiträge, kurze Mitteilungen, Übersichtsartikel und Sonderhefte über die Physiologie, Anatomie, Chemie, Pharmakologie und Pathologie der Gliazellen. Die Zeitschrift erscheint im Verlag Wiley-Liss, New York (USA), die Herausgeber sind Bruce Ransom und Helmut Kettenmann.

Chinese-German Laboratory for Molecular Medicine in Beijing

The identification of genes which are involved in causing cardiovascular and associated diseases is the aim of the Chinese-German Laboratory for Molecular Medicine which has recently been set up in Beijing by the Fu Wai Hospital of the Chinese Academy of Medical Sciences (CAMS) and the MDC. This laboratory includes a "Genome Screening Center" which was set up in 1999 with funds from the German Federal Research Ministry (German abbrev. BMBF). There, scientists are trying to identify the genes that are responsible for these diseases. This has led to more intensive collaboration between researchers at the CAMS and the MDC. From November 2000, the Beijing Laboratory for Molecular Medicine (headed by Hui Rutai and Liu Lisheng) has been assisted by the activities of two "Young Investigator Partnership Groups". The laboratory in the Fu Wai Hospital is supported by the CAMS, the Chinese Ministry of Research, the MDC and the Swiss pharmaceutical company, Hoffmann-La Roche, in Basle. A number of other groups are also taking part in this research including the Chinese Human Genome Project and the Franz Volhard Clinic for Cardiovascular Diseases (Charité University Clinic on the Berlin-Buch Campus). Fellowships to support guest scientists in Beijing and Berlin are provided by the Society of Friends and Sponsors of the MDC. The Fu Wai Hospital, a specialist facility for cardiovascular diseases on western lines, also acts as a reference center for these diseases in China. Since 1995, the MDC has provided a center for gene mapping. This specialist facility is headed by Norbert Hübner and Peter Nürnberg and is funded by the BMBF under its German Human Genome Project.

Chinesisch-deutsches Labor für Molekulare Medizin in Peking

Die Identifizierung von Genen, die an der Entstehung von Herz-Kreislauferkrankungen und ihren Folgekrankheiten beteiligt sind, ist das Ziel des chinesisch-deutschen Labors für Molekulare Medizin, welches das Fu Wai Krankenhaus der Chinesischen Akademie der Medizinischen Wissenschaften (CAMS) und das MDC in Peking in den vergangenen Jahren aufgebaut haben. Zu diesem Pekinger Labor gehört ein 1999 mit Mitteln des Bundesforschungsministeriums (BMBF) eingerichtetes „Genom-Screening-Zentrum", in dem die Forscher systematisch nach Genen fahnden, die Krankheiten verursachen. Damit konnte die Zusammenarbeit zwischen Wissenschaftlern der CAMS und dem MDC intensiviert werden. Seit November 2000 ist das Pekinger Labor für Molekulare Medizin (Leitung Hui Rutai und Liu Lisheng) am Fu Wai Hospital durch zwei junge Forschergruppen („Young Investigator Partnership Groups") verstärkt worden. Das Labor im Fu Wai Hospital wird gefördert von der CAMS, dem chinesischen Forschungsministerium, dem MDC sowie dem Schweizer Pharmakonzern Hoffmann-La Roche in Basel. An den Forschungsprojekten beteiligt sind auch das Chinesische Humangenomprojekt und die Franz-Volhard-Klinik für Herz-Kreislauferkrankungen (Universitätsklinikum Charité, Campus Berlin-Buch). Mit Stipendien für Forschungsaufenthalte von Gastwissenschaftlern in Peking und Berlin unterstützt der Verein der Freunde und Förderer des MDC diese Initiative. Das Fu Wai Krankenhaus, eine nach westlicher Medizin ausgerichtete Spezialklinik für Herz-Kreislauf-Erkrankungen, ist zugleich ein Referenzzentrum für diese Erkrankungen in China. Das MDC verfügt seit 1995 über ein Zentrum für Genkartierung. Dieses von Norbert Hübner und Peter Nürnberg geleitete Speziallabor wird im Rahmen des Deutschen Humangenomprojekts vom BMBF gefördert.

Congresses

In the years reported, two major conferences took place in Berlin which were organized by scientists from the MDC.

International Conference on "Cell Migration in Development and Disease"

Cell migration is an important process in development. Frequently precursor cells are generated at one site in the embryo and then migrate over long distances to targets where they differentiate. Migration processes in the embryo are well controlled, and migrating cells are generated during distinct stages and use characteristic and even invariant paths to reach their targets. Ordered migration of cells can also be observed in the adult, e.g. in the immune system. Such well controlled processes are in contrast to the aberrant migration of cells in pathological processes such as cancer.

Important recent insight into these processes was provided at an international conference on "Cell Migration in Development and Disease" (November 29 to December 1, 2001), which was held by the MDC in Berlin. The conference was funded by the Deutsche Forschungsgemeinschaft (DFG; German Research Fondation) and the MDC. More than 200 scientists from the USA, Australia, Israel, Japan, and many

Kongresse

In den vergangenenen zwei Jahren fanden zwei wichtige Konferenzen in Berlin statt, die von Wissenschaftlern des MDC organisiert wurden.

Internationaler Kongress zu „Cell Migration in Development and Disease"

Die Fähigkeit von Zellen, während der Entwicklung eines Organismus zu wandern, ist ein lebenswichtiger Prozess. Sehr häufig entstehen in einem Embryo so genannte Vorläuferzellen, die dann zu weit entfernten Zielorganen wandern müssen, wo sie differenzieren, das heißt, zu ganz spezifischen Körperzellen werden. Diese Wanderungsprozesse von Zellen im Embryo sind sehr genau gesteuert. Die Fähigkeit von Zellen, ihren Entstehungsort zu verlassen, wird in bestimmten Entwicklungsphasen des Organismus benötigt. Sie nutzen ganz charakteristische, vorgebene und sich niemals ändernde Pfade, um ihre Zielpunkte zu erreichen. Eine geordnete Zellwanderung wird auch im erwachsenen Organismus beobachtet, zum Beispiel im Abwehrsystem des Körpers, dem Immunsystem. Gerät die Zellwanderung aus dem Takt, dann können Krankheiten, wie zum Beispiel Krebs, entstehen.

European countries attended. The organisers were Walter Birchmeier (MDC), Doris D. Wedlich (Technical University of Karlsruhe, Germany), Carmen Birchmeier and Martin Lipp (both MDC).

2nd International Symposium on Obesity and Hypertension

Overweight and obesity currently affect approximately 100 million adults in the countries of the European Union and in most European countries more than 20 per cent of the population are obese. In Germany, more than 16 million people are obese while more than 40 million, i.e. half the population, is overweight. Obesity is now recognized as an important risk factor for a number of medical problems including cardiovascular diseases, such as hypertension, and diabetes. Healthcare related costs are around 200 times higher for obese individuals, says Arya Sharma, physician and nephrologist at the Franz Volhard Clinic (FVK) for Cardiovascular Diseases of the Charité, Medical School of the Humboldt University of Berlin. In recent years, there have been remarkable advances in our understanding of the biology of fat (adipose) tissue. Fat cells (adipocytes) are now seen as an important source of a variety of molecules that can affect blood pressure regulation and kidney function. Scientists and physicians hope that these findings will eventually lead to new preventive strategies and therapies for managing obesity and related cardiovascular diseases. These diseases were the key topics of the 2nd International Symposium on Obesity and Hypertension held in Berlin from October 25 to October 27, 2001 at the MDC. 200 scientists and physicians from the USA, Canada, Japan, Australia, Israel and Europe attended this symposium. The organisers were Arya Sharma, Friedrich Luft (FVK and MDC) and Achim Leutz (MDC). This symposium was officially endorsed by the European Society of Hypertension, the European Association for the Study of Obesity and the International Obesity Task Force. It was supported by the Deutsche Forschungsgemeinschaft, and by the "High-Level Scientific Meeting Programme" of the European Union.

Academic Appointments

Six new group leaders have been appointed during the last two years (2000 and 2001).

The biologist *Christiane Alexander* was appointed head of the research group "Neurodegeneration" in the Neurosciences section of the MDC for a period of five years in July 2001. She received her degree from the University of Düsseldorf in 1994 and her doctorate four years later. Her postdoctoral research involved molecular genetics, first one year at Tübingen University Clinic, followed by two years at University College, London, supported by a DFG-Postdoctoral scholarship.

Gerd Kempermann was appointed head of the "Neuronal Stem Cells" research group at the MDC in September 2000. He completed his medical studies in Cologne and Freiburg im Breisgau with a practical year in New York in 1993. In the same year, he obtained his doctorate in the Department of Neuropathology, Pathology Institute, University of Freiburg. He spent a period of two years practical training as a physician. He then obtained a DFG scholarship for three years at

Mit diesen Prozessen befasste sich ein Internationaler Kongress „Cell Migration in Development and Disease" (Zellwanderung in der Entwicklung von Organismen und bei der Entstehung von Krankheiten), den das MDC vom 29. November bis 1. Dezember 2001 in Berlin veranstaltet hatte. Der Kongress wurde unterstützt von der Deutschen Forschungsgemeinschaft (DFG) und dem MDC. Über 200 Wissenschaftler aus den USA, Australien, Israel, Japan und mehreren europäischen Ländern nahmen an dem Kongress teil. Organisatoren waren Walter Birchmeier (MDC), Doris D. Wedlich (Technische Universität Karlsruhe), Carmen Birchmeier und Martin Lipp (beide MDC).

2. Internationales Symposium zu Fettsucht und Bluthochdruck

Etwa 100 Millionen Erwachsene in den Ländern der Europäischen Union sind übergewichtig oder zu dick. Allein in der Bundesrepublik bringen 40 Millionen Menschen, und damit nahezu die Hälfte der Bevölkerung, nach Ansicht von Medizinern zuviel Gewicht auf die Waage. 20 Prozent, rund 16 Millionen Menschen, gelten als adipös. Menschen mit Übergewicht und Fettsucht sind häufig auch chronisch krank. Sie sind anfällig für Herz-Kreislauf-Erkrankungen, zum Beispiel Bluthochdruck (Hypertonie), sowie für Diabetes. Sie verursachen nach Aussage von Arya Sharma (Franz-Volhard-Klinik (FVK) der Charité, Humboldt-Universität zu Berlin, Campus Berlin-Buch) darüber hinaus 200 mal höhere Gesundheitskosten als schlanke Menschen. In den vergangenen Jahren haben Grundlagenforscher sehr viele neue Erkenntnisse über die Biologie des Fettgewebes gewonnen. So wissen sie inzwischen, dass eine Vielzahl von Molekülen, die von Fettzellen produziert werden, direkt das Herz-Kreislaufsystem und die Nieren schädigen können. Fettsucht und Bluthochdruck standen im Mittelpunkt des 2. internationalen Symposiums für Grundlagenforscher und Kliniker, das Arya Sharma gemeinsam mit Friedrich Luft (FVK und MDC) und Achim Leutz (MDC) in Berlin-Buch organisiert hatte. An dem Symposium vom 25. Oktober bis 27. Oktober 2001 hatten rund 200 Wissenschaftler aus den USA, Kanada, Japan, Australien, Israel und Europa teilgenommen. Es wurde unterstützt von der Europäischen Gesellschaft für Bluthochdruck, der Europäischen Vereinigung für die Erforschung der Adipositas, der Internationalen Adipositas Task Force sowie der Deutschen Forschungsgemeinschaft (DFG) und dem „High-Level Scientific Conference Programme" der Europäischen Union.

Akademische Berufungen

Sechs neue Gruppenleiter sind in den beiden vergangenen Jahren (2000 und 2001) berufen worden :

Die Biologin *Christiane Alexander* leitet seit Juli 2001 für fünf Jahre die Forschungsgruppe „Neurodegeneration" im Bereich Neurowissenschaften am MDC. 1994 legte sie an der Universität Düsseldorf die Diplomprüfung ab und promovierte dort vier Jahre später. In ihrer Postdoc-Zeit beschäftigte sie sich mit molekularer Genetik, zuerst für ein Jahr am Universitätsklinikum Tübingen, anschließend zwei Jahre am University-College London/Großbritannien, finanziert mit einem DFG-Postdoc-Stipendium.

the Genetics Laboratory of the Salk Institute, La Jolla, California. In October 1998, he was appointed a scientific assistant at the Regensburg Neurological University Clinic before coming to the MDC.

Peter Nürnberg was appointed head of the research group "Molecular Genetics and Center for Gene Mapping" at the MDC for a period of five years in February 2001. He studied biology until 1981 at the Humboldt University of Berlin. After carrying out research at the Institute of Biochemistry, Charité, and the Central Institute of Molecular Biology in Berlin-Buch, he obtained his doctorate in 1984. He has also worked at the Institute of Medical Genetics, Charité, where he was appointed head of the Molecular Genetics Department in 1989, and the Max Planck Institute of Psychiatry, Martinsried. In 1997, he qualified as a university lecturer in human genetics and was appointed senior assistant in September 1998.

Erich Wanker was appointed head of the "Proteome Research and Molecular Mechanisms of Neurodegenerative Diseases" research group following a C4-grade appointment in "Molecular Medicine" at the Humboldt University of Berlin and the MDC, in September 2001. He is also responsible for the core facility "Proteomics" at the MDC. The Austrian-born scientist studied biochemistry at the Technical University in Graz, obtaining his doctorate in 1992. After a three-year post-doctoral fellowship at the University of California in Los Angeles, he led the "Huntington's Disease Project" at the Max Planck Institute of Molecular Genetics in Berlin-Dahlem. In 1999, he was appointed to a C3-grade post there before coming to the MDC.

Salim Abdelilah-Seyfried has been head of the research group "Cell Polarity and Epithelium Formation" since September 2001. After obtaining his high school leaving certificate in Düsseldorf and studying biochemistry, molecular biology and zoology in Manchester, Tübingen and Harvard Medical School, Boston/USA, the Algerian-born scientist graduated in biology at the University of Tübingen. He was interested in the Zebra fish as a model for genetic research and obtained his doctorate in 1996 from the Faculty of Biology, University of Tübingen. After a short period at the Max Planck Institute of Biochemistry in Martinsried, he undertook postdoctoral research at the University of California in San Francisco, before moving to the MDC for a period of 5 years.

Christof Tannert is principal investigator in a project "Discourse to the Ethical Questions of Biomedicine", funded by the Federal Ministry for Education and Research, and has headed a group "Bioethics and Scientific Communication" at the MDC since January 2002. He studied biology at the University of Leipzig. He then carried out research at an Academy Institute in Leipzig, became an assistant at the Charité, Humboldt University, Berlin, studying the destruction of mitochondria and lipid peroxidation as well as the biochemistry of erythrocyte aging, and obtained his doctorate in biology in 1979. From 1980 to 1984, he studied theology by enrolling in a distance-learning course in the diocese of Saxony and then worked freelance on questions involving scientific ethics. In 1987, he started the Independent Institute for Environmental Problems, Berlin/Halle, which he headed from 1989 to 1993.

Gerd Kempermann ist im September 2000 für fünf Jahre als Leiter der Forschungsgruppe „Neuronale Stammzellen" an das MDC berufen worden. Er studierte Medizin in Köln und Freiburg i. Br. und schloß es mit einem praktischen Jahr in New York 1993 ab. Im gleichen Jahr verteidigte er seine Promotion in der Abteilung Neuropathologie des Pathologischen Institutes der Universität Freiburg. Dort arbeitete er auch zwei Jahre als Arzt im Praktikum. Anschließend ging er mit einem Ausbildungsstipendium der DFG für drei Jahre an das Genetische Labor des Salk Instituts, La Jolla, Kalifornien/USA. Im Oktober 1998 hatte er die Stelle eines wissenschaftlichen Assistenten an der Neurologischen Universitätsklinik Regensburg erhalten, bevor er ans MDC kam.

Peter Nürnberg leitet seit Februar 2001 für den Zeitraum von fünf Jahren die Forschungsgruppe „Molekulare Genetik und Zentrum für Genkartierung". Er studierte bis 1981 an der Humboldt-Universität zu Berlin Biologie. Nach Forschungsarbeiten am Institut für Biochemie der Charité und am Zentralinstitut für Molekularbiologie in Berlin-Buch promovierte er 1984. Stationen seiner weiteren wissenschaftlichen Laufbahn waren neben Berlin-Buch, das Institut für Medizinische Genetik der Charité der Humboldt-Universität zu Berlin, an dem er ab 1989 die Abteilung „Molekulargenetik" leitete, und das Max-Planck-Institut für Psychiatrie, Martinsried. 1997 hatte er sich für das Fach Humangenetik habilitiert.

Erich Wanker hat im September 2001 von der Humboldt-Universität zu Berlin und dem MDC einen Ruf als C4-Professor auf den Lehrstuhl „Molekulare Medizin" sowie als Forschungsgruppenleiter „Proteomforschung und molekulare Mechanismen der neurodegenerativen Erkrankungen" erhalten. Am MDC leitet er zudem die Core-facility „Proteomics". Der in Österreich geborene Wissenschaftler studierte Biochemie an der Technischen Universität in Graz, wo er 1992 auch promovierte. Nach einer dreijährigen Postdoc-Zeit an der Universität von Kalifornien in Los Angeles leitete er am Max-Planck-Institut für Molekulare Genetik in Berlin-Dahlem das „Huntington's Disease Project". 1999 wurde er dort in eine C3-Position berufen.

Salim Abdelilah-Seyfried leitet seit September 2001 die MDC-Forschungsgruppe „Zellpolarität und Epithelbildung". Nach Abitur in Düsseldorf und Studien der Biochemie, Molekularen Biologie und Zoologie in Manchester/Großbritannien, Tübingen und an der Harvard Medical School in Boston/USA, legte der in Algerien geborene Wissenschaftler 1993 seine Diplomprüfung in Biologie an der Universität Tübingen ab. Er beschäftigte sich mit dem Zebrafisch als Modell genetischer Untersuchungen und promovierte 1996 an der Fakultät für Biologie der Universität Tübingen. Als Postdoc ging er nach kurzem Aufenthalt am Max-Planck-Institut für Biochemie in Martinsried, an die Universität von Kalifornien/USA in San Francisco. Von dort wurde er für 5 Jahre an das MDC berufen.

Christof Tannert verantwortet ein vom BMBF gefördertes Projekt „Diskurs zu den ethischen Fragen der Biomedizin" und leitet seit Januar 2002 am MDC die Arbeitsgruppe „Bioethik und Wissenschaftskommunikation". Er studierte an der Universität Leipzig Biologie und arbeitete danach in einem Leipziger Akademie-Institut und an der Charité der

From 1990 to 1994, he was a member of the Berlin State Parliament and, from 1994 to 1999, a member of the European Parliament in Strasbourg/France.

Further appointments at the MDC

Thomas Willnow received a tenured position as research group leader at the MDC in February 2001. A joint C4-grade appointment with the Free University of Berlin in Molecular Cardiovascular Research was made in September of that same year. He studied biology in Munich and obtained his doctorate in 1991. He then worked at the Southwestern Medical Center in Dallas/USA. He received a Heisenberg-Stipend and has been research group leader at the MDC since 1996. He succeeded Ernst-Georg Krause, who had headed the research group "Intracellular Signal Transduction in the Cardiovascular System" until his retirement in December 2000.

Wolfgang Uckert has been offered a joint C3-grade professorship in "Molecular Cell Biology and Gene Therapy" at the MDC and the Humboldt University, Berlin.

Appointments of MDC scientists to other scientific Institutions

Young-Ae Lee from the MDC Molecular Genetics and Gene Mapping Center has been appointed to a junior professorship in pediatrics for an initial period of 3 years at the Charité Medical School of the Humboldt-University of Berlin. She received her doctorate in 1999. Her research focusses on the analysis of complex genetic diseases.

André Wiesmann Da Silva Reis, research group leader of MDC's Molecular Genetics and Gene Mapping Center, was appointed to the Chair of Human Genetics at the Friedrich Alexander University Erlangen-Nürnberg in June 2000.

Jürgen Behrens from the research group "Epithelial Differentiation, Invasion and Metastasis" accepted a C4-grade professorship in Experimental Medicine (Molecular Tumor Research) in September 2000 at the Friedrich Alexander University Erlangen-Nürnberg.

Reinhold Förster from the research group "Molecular Tumor Genetics" was appointed to a grade C3 chair in Experimental Surgery at the University Erlangen-Nürnberg in January 2001.

Frank W. Pfrieger, research group leader "Formation and Function of Synapses", was appointed to a 5-year post to head a junior group at the Institute of Genetics and Molecular and Cellular Biology in Strasbourg/France. It is jointly funded by the French CNRS and the the Max Planck Society starting in January 2001.

Andreas Schedl, research group leader "Developmental Genetics in the Cardiovascular System", was appointed to a professorship in murine genetics at the Institute of Human Genetics at the International Centre for Life in Newcastle upon Tyne/United Kingdom in March 2001.

Hans-Dieter Royer, research group leader "Cell Cycle Regulation", was appointed group leader and chief physician at the Institute of Transplantation Diagnosis and Cell Therapeutics at the University of Düsseldorf in December 2000.

Humboldt-Universität zu Berlin. 1979 promovierte er im Fach Biologie. Von 1980 bis 1984 studierte er in einem Fernkurs der Kirchenprovinz Sachsen Theologie und arbeitete danach freiberuflich über Fragen der Wissenschaftsethik. 1987 initiierte er das Unabhängige Institut für Umweltfragen Berlin/Halle, das er von 1989 bis 1993 leitete. Von 1990 bis 1994 war er Mitglied des Berliner Abgeordnetenhauses sowie von 1994 bis 1999 Mitglied des Europäischen Parlaments in Straßburg/Frankreich.

Weitere Berufungen im MDC

Thomas Willnow ist im Februar 2001 als unbefristeter Forschungsgruppenleiter an das MDC berufen worden. Die gemeinsame Berufung mit der Freien Universität Berlin auf die C4-Position für das Fachgebiet Molekulare Herz-Kreislauf-Forschung erfolgte im September des gleichen Jahres. Er trat damit die Nachfolge von Ernst-Georg Krause an. Thomas Willnow studierte Biologie in München. Nach seiner Promotion 1991 folgte ein mehrjähriger Forschungsaufenthalt am Southwestern Medical Center in Dallas/USA. Der Heisenberg Stipendiat hatte 1996 eine Forschungsgruppe am MDC erhalten.

Wolfgang Uckert von der MDC-Forschungsgruppe „Molekulare Immunologie und Gentherapie" hat im Frühjahr 2002 einen Ruf auf eine gemeinsame C 3-Professur „Molekulare Zellbiologie und Gentherapie" am MDC und der Humboldt-Universität zu Berlin erhalten.

Berufungen von MDC-Wissenschaftlern an andere wissenschaftliche Einrichtungen

Young-Ae Lee vom Zentrum für Genkartierung des MDC und der Kinderklinik der Charité der Humboldt-Universität zu Berlin hat den Ruf auf eine vorerst auf 3 Jahre befristete Stelle der Juniorprofessur des Faches Kinderheilkunde an der Charite. Sie hatte 1999 an der Freien Universität (FU) Berlin promoviert. Ihr Forschungsschwerpunkt ist die genetische Analyse multifaktorieller Erkrankungen.

André Wiesmann Da Silva Reis, Forschungsgruppenleiter des Zentrum für Genkartierung des MDC hat den Ruf auf den Lehrstuhl für Humangenetik der Friedrich-Alexander-Universität Erlangen-Nürnberg im Juni 2000 angenommen.

Jürgen Behrens, Forschungsgruppe „Epitheliale Differenzierung, Invasivität und Metastasierung", hat im September 2000 den Ruf an die Friedrich-Alexander-Universität Erlangen-Nürnberg als C 4-Professor für Experimentelle Medizin (Molekulare Tumorforschung) angenommen.

Reinhold Förster, Forschungsgruppe „Molekulare Tumorgenetik und Immungenetik", hat im Januar 2001 den Ruf als C 3-Professor auf den Lehrstuhl für Experimentelle Chirurgie, an die Universität Erlangen-Nürnberg angenommen .

Frank W. Pfrieger, Forschungsgruppenleiter „Bildung und Funktion von Synapsen", hat ab Januar 2001 eine auf 5 Jahre befristete Anstellung bei der Max-Planck-Gesellschaft erhalten, um im Rahmen eines Austauschprogramms eine Nachwuchsgruppe des CNRS am Institut de Genetique et de Biologie Moleculaire et Cellulaire in Strasbourg/Frankreich zu leiten.

Heinrich Leonhardt research group leader "Cell Biology of Cardiovascular Diseases" was appointed to a grade C3 university professorship in Molecular Human Genetics at the Ludwig Maximilian University, Munich, starting April 1, 2002.

Retirements

Brigitte Wittmann-Liebold, research group leader "Protein Chemistry and Proteomic Analysis" since 1992 remained active although reaching retirement age until her group was taken over by Erich Wanker at the end of 2001. Her group collaborated closely with and received financial support from the Max Planck Society.

Dietrich Arndt, research group leader "Phospholipids", retired from his scientific activities at the MDC on reaching retirement age in December 2001. He had a PhD in chemistry and had worked at the Buch research institutes since 1972.

Uwe Karsten, head of the research group "Tumor-associated Glycoconjugates" will have complete his scientific work in Berlin-Buch at the end of 2002. He has a PhD in biology and worked in a number of the Academy's institutes on the campus since 1967 .

Ernst-Georg Krause retired in December 2000 as head of the MDC research group "Intracellular Signal Transduction in the Cardiovascular System". His successor is Thomas Willnow. After obtaining his doctorate and qualifying as a university lecturer, he was appointed a professor in 1979 at the Humboldt University of Berlin. He had worked on the Berlin Campus since 1958 and headed the research group "Intracellular Signal Transduction in the Cardiovascular System" until his retirement. He is a member of the renowned Deutsche Akademie der Naturforscher Leopoldina in Halle (German Academy of Natural Scientists Leopoldina).

Awards

A number of prestigious prizes have been awarded to scientists of the MDC and clinicians of the collaborating university affiliated Robert Rössle Cancer Clinic and Franz Volhard Clinic for Cardiovascular Diseases in 2000 and 2001.

Leibniz Prize awarded to Carmen Birchmeier

Developmental biologist and gene researcher Carmen Birchmeier, research group leader at the MDC, has won the most valuable award of the German Research Society (Deutsche Forschungsgemeinschaft, DFG), the Gottfried Wilhelm Leibniz Prize. The prize is awarded for a period of five years. and is worth 1.55 million Euro (3 million Deutsch marks). Carmen Birchmeier's main research interests center on molecular biology and mammalian embryo and organ development. She is particularly interested in signal transmission which is of critical importance for communication between cells during development. The ability of cells to maintain a continuous dialogue with each other and coordinate their functions is also fundamentally important for sustaining all the key functions that are essential to human and animal life. A breakdown in the communication pathways can lead to severe developmental problems in the embryo and result in serious

Andreas Schedl, Forschungsgruppenleiter „Entwicklungsgenetik des Herz-Kreislauf-Systems", hat im März 2001 einen Ruf auf die Professur für Mausgenetik am Institute of Human Genetics des International Centre for Life in Newcastle upon Tyne/Grossbritannien angenommen.

Hans-Dieter Royer, Forschungsgruppenleiter „Regulation des Zellzyklus", hat im Dezember 2000 die Stelle eines Arbeitsgruppenleiters und leitenden Oberarztes am Institut für Transplantationsdiagnostik und Zelltherapeutika der Universität Düsseldorf angetreten.

Heinrich Leonhardt, Forschungsgruppe „Zellbiologie kardiovaskulärer Erkrankungen", hat im Juni 2001 einen Ruf als C3-Universitätsprofessor für Molekulare Humangenetik an die Ludwig-Maximilians-Universität München erhalten und ihn zum 1. April 2002 angenommen.

Emeritierungen

Brigitte Wittmann-Liebold leitete seit 1992 die Arbeitsgruppe „Proteinchemie und Proteomanalyse", die in enger Kooperation und unter Förderung der Max-Planck-Gesellschaft tätig wurde. Auch nach ihrer Emeritierung war sie am MDC bis zur Übernahme der Arbeitsgruppe durch Erich Wanker, Ende des Jahres 2001, wissenschaftlich aktiv.

Dietrich Arndt, Leiter der MDC-Arbeitsgruppe „Phospholipide", hat nach Erreichen des Rentenalters seine wissenschaftliche Tätigkeit am MDC im Jahre 2002 beendet. Der promovierte Chemiker hatte seit 1972 in Bucher Forschungsinstituten gearbeitet.

Uwe Karsten hat mit Ablauf des Jahres 2002 seine wissenschaftliche Tätigkeit in Berlin-Buch beendet. Er leitete die Arbeitsgruppe „Tumorassoziierte Glykokonjugate". Als promovierter Biologe arbeitete er seit 1967 auf dem Wissenschaftscampus in verschiedenen Akademieinstituten und schließlich am MDC in der Krebsforschung.

Ernst-Georg Krause, Leiter der Forschungsgruppe „Intrazelluläre Signalumsetzung im Herz-Kreislauf-System", wurde am 31. Dezember 2000 emeriert. Er war seit 1958 auf dem Bucher Campus wissenschaftlich tätig. Nach Promotion und Habilitation an der Humboldt-Universität zu Berlin, wurde er 1979 zum Professor berufen. Ernst-Georg Krause ist Mitglied der Deutschen Akademie der Naturforscher Leopoldina in Halle. Sein Nachfolger ist Thomas Willnow.

Preise

Eine Reihe angesehener Preise wurde in den Jahren 2000 und 2001 Wissenschaftlern des MDC und der kooperierenden universitären Kliniken zuerkannt.

Leibniz-Preis für Carmen Birchmeier

Mit dem höchstdotierten Preis der deutschen Wissenschaft, dem Gottfried Wilhelm Leibniz-Preis der Deutschen Forschungsgemeinschaft (DFG), ist für das Jahr 2002 die Entwicklungsbiologin und Genforscherin Carmen Birchmeier vom MDC ausgezeichnet worden. Sie erhielt für die Dauer von fünf Jahren 1,55 Millionen Euro (drei Millionen Mark).

conditions in the fully developed animal. Carmen Birchmeier is engaged in the study of the function of a range of growth factors. The internationally renowned researcher was able to identify some of these factors as key role players in the development of the nervous system and heart.

Berlin-Buch and Munich scientists win the Erwin Schrödinger Prize worth 100,000 Deutsch marks

Martin Lipp (MDC), Reinhold Förster (formerly MDC, currently professor at the Medical University, Hannover), Elisabeth Kremmer (GSF-Research Center for Health and the Environment, Neuherberg) and Eckhard Wolf (Gene Center, Ludwig Maximilian University, Munich) have been awarded the Erwin Schrödinger Prize 2000 for their discovery of the fundamental mechanism that allows immune cells to track down and destroy pathogens and which may also play a role in autoimmune diseases and the rejection of transplanted organs. The prize was established by the Donors' Association for German Science (Stifterverband für die Deutsche Wissenschaft) in 1999 and is awarded by the Helmholtz Association for interdisciplinary research.

Friedrich Luft wins the Lingen Prize

Friedrich Luft was awarded the Prize from the Helmut and Ruth-Lingen Foundation (Cologne), worth 100,000 Deutsch marks on November 30, 2001, for his "outstanding efforts in the pathophysiology, clinical aspects and treatment of renal and vascular disease". Luft is an nephrologist at the Franz Volhard Clinic for Cardiovascular Diseases at the Charité, Humboldt University Berlin, and also leads a research group at the Max-Delbrück Center for Molecular Medicine (MDC) Berlin-Buch. He intends to use his prize money to support young researchers.

Domagk Prize for Jürgen Behrens

Jürgen Behrens (formerly of the MDC, currently professor at the University of Erlangen) has been awarded the Gerhard Domagk Prize for his "ground-breaking research" on the etiology of cancer. He received the prize worth 20,000 Deutsch marks on June 5, 2000, at the University of Münster. Since 1963, the prize has been awarded biannually. Behrens and his group have discovered a number of control molecules which, when they undergo alteration, play an important role in the etiology of intestinal cancer because cellular signal cascades are not under proper control.

Young-Ae Lee receives the SmithKline Beecham Prize

The prize for clinical research, worth 25,000 Deutsch marks, donated by the SmithKline Beecham Foundation was awarded in 2001 to Young-Ae Lee who works at the MDC and the Charité Children's Clinic of the Humboldt University Berlin. The pediatrician and researcher received the prize on April 24, 2001, in Wiesbaden for her research into neurodermitis, one of the most common chronic diseases of childhood. In a study involving countries across Europe she collaborated with researchers from Germany, Italy, Sweden and the Netherlands and identified for the first time a region on chromosome 3 which contains the neurodermitis gene. A significant system involved in a predisposition to allergies has been traced to this same site on chromosome 3.

Carmen Birchmeier befasst sich vor allem mit molekularbiologischen Fragen der Embryonal- und Organentwicklung der Säuger. Dabei erforscht sie insbesondere die Signalübertragung, die für ein geordnetes Zusammenspiel von Zellen während dieser Entwicklungsperioden eine wichtige Rolle spielen. Die Fähigkeit von Zellen, in ständigem Dialog miteinander zu stehen und ihre Funktionen aufeinander abzustimmen, ist auch für die Aufrechterhaltung der Lebensfunktionen von Mensch und Tier von grundlegender Bedeutung. Sind die fein aufeinander abgestimmten Signalwege gestört, kann es im Embryo zu Entwicklungsstörungen und im erwachsenen Organismus zur Entstehung schwerer Krankheiten kommen.
Carmen Birchmeier und ihrer Forschungsgruppe ist es gelungen, die Rolle einer Reihe von Wachstumsfaktoren bei der Entwicklung des Organismus aufzuklären. Einige von ihnen sind entscheidend für die Entstehung von Brustkrebs oder lösen Fehlfunktionen des Nervensystems und des Herzens aus.

Berlin-Bucher und Münchner Wissenschaftler erhalten den mit 100.000 Mark dotierten Erwin Schrödinger-Preis

Für die Aufklärung eines grundlegenden Mechanismus, durch den Immunzellen ihre Fähigkeit erhalten, Krankheitserreger aufzuspüren und zu vernichten und der auch bei Autoimmunerkrankungen und der Abstoßung von Organtransplantaten eine Rolle spielen könnte, haben Martin Lipp (MDC), Reinhold Förster (früher MDC, jetzt Professor an der Medizinischen Hochschule Hannover), Elisabeth Kremmer (GSF-Forschungszentrum für Umwelt und Gesundheit, Neuherberg) und Eckhard Wolf (Gen-Zentrum der Ludwig-Maximilians-Universität München) den Erwin-Schrödinger-Preis 2000 erhalten. Der vom Stifterverband für die Deutsche Wissenschaft initiierte Preis wird seit 1999 von der Helmholtz-Gemeinschaft für interdisziplinäre Forschung vergeben.

Friedrich Luft erhält Lingen-Preis

Für seine „herausragenden Leistungen auf dem Gebiet der Pathophysiologie, Klinik und Therapie der Nieren- und Gefäßerkrankungen" hat Friedrich Luft am 30. November 2001 den mit 100.000 Mark dotierten Preis der Helmut und Ruth-Lingen Stiftung (Köln) erhalten. Luft ist Nephrologe an der Franz-Volhard-Klinik für Herz-Kreislauf-Erkrankungen der Charité der Humboldt-Universität zu Berlin und leitet darüber hinaus eine Forschungsgruppe am Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch. Mit dem Preisgeld will er junge Wissenschaftler fördern.

Domagk-Preis an Jürgen Behrens

Für seine „bahnbrechenden Forschungen" über die Entstehung von Krebs ist Jürgen Behrens (damals MDC, jetzt Professor an der Universität Erlangen) mit dem Gerhard-Domagk-Preis ausgezeichnet worden. Er erhielt den mit 20.000 Mark dotierten Preis am 5. Juni 2000 in der Universität Münster überreicht. Der Preis wird seit 1963 alle zwei Jahre verliehen. Behrens und Mitarbeiter entdeckten verschiedene Kontrollmoleküle, deren Veränderung bei der Entstehung von Darmkrebs eine wichtige Rolle spielt, weil zelluläre Signalkaskaden fehlreguliert werden.

SmithKline Beecham Prize for Thomas Willnow

Thomas Willnow, a research group leader at the MDC, has won the Basic Medicine Research Prize 2000 awarded by the SmithKline Beecham Foundation for his research into the metabolism of Vitamin D. He has shown how Vitamin D, a steroid hormone which is essential for healthy bone growth, is taken up from the bloodstream and stored in the kidneys where it can be activated. The results of his research suggest how other steroid hormones in the body reach their target sites, including those that promote the growth of certain breast and prostate tumors. The prize is worth 25,000 Deutsch marks and was presented to him on May 3, 2000, in Wiesbaden.

Sofja Kovalevskaja Prize for Michael Gotthardt

Physician Michael Gotthardt (32) is one of 29 trainee researchers who received the new and valuable Sofja Kovalevskaja Prize of the Alexander von Humboldt Foundation, to allow them to set up their own research groups in Germany. Several years ago he worked at the MDC in the group led by Prof. Michael Strauss, before going to the USA. There he worked at the Universities of Dallas (Texas) and Pullman (Washington). With the help of the prize fellowship worth 1.2 million Euro, he returned to the MDC in 2002. The program provides support for a period of three years.

MDC and the Karlsruhe Research Center win the first technology transfer prize of the Federal Research Ministry

The 400,000 Deutsch mark “Prize of the Federal Ministry for formation and research for start-up initiatives, awarded by the Karl Heinz Beckurts Foundation” is divided equally between the MDC and the Karlsruhe Research Center. This is the first time the prize has been awarded and the Federal Research Ministry (Bundesministerium für Bildung und Forschung, BMBF) wishes to support initiatives by extra-university research institutions to develop new commercial ideas and encourage the setting up of technology-based companies. The prize was awarded to the two members of the Helmholtz Association of Helmholtz Association of National Research Laboratories (Helmholtz-Gemeinschaft Deutscher Forschungszentren, HGF) on December 8, 2000, during a ceremony held by the Beckurts Foundation in Munich by the Parliamentary Secretary of State to the Federal Research Ministry, Wolf-Michael Catenhusen. The MDC received the prize for “Setting up a network of biomedical companies and research facilities for venture capital companies leading to a large number of companies establishing themselves on the campus”.

Young-Ae Lee erhielt SmithKline Beecham Preis

Der mit 25 000 Mark dotierte Preis für klinische Forschung der SmithKline Beecham Stiftung ist im Jahr 2001 an Young-Ae Lee vom MDC und der Charité-Kinderklinik der Humboldt-Universität zu Berlin gegangen. Die Kinderärztin und Wissenschaftlerin erhielt den Preis am 24. April 2001 in Wiesbaden für ihre Forschungen über die Neurodermitis, eine der häufigsten chronischen Krankheiten im Kindesalter. In einer europaweiten Studie hatte sie gemeinsam mit Wissenschaftlern aus der Bundesrepublik, Italien, Schweden und den Niederlanden erstmals eine Genregion auf Chromosom 3 identifiziert, die ein Krankheitsgen der Neurodermitis enthält. Eine erbliche Anlage zur Allergieneigung konnte an demselben Genort auf Chromosom 3 nachgewiesen werden.

SmithKline Beecham Preis für Thomas Willnow

Für die Erforschung des Vitamin D-Stoffwechsels ist Thomas Willnow, Forschungsgruppenleiter im MDC, mit dem Preis für medizinische Grundlagenforschung 2000 der SmithKline Beecham Stiftung ausgezeichnet worden. Er hatte nachgewiesen, auf welche Weise Vitamin D, ein Steroidhormon, das für gesundes Knochenwachstum unerlässlich ist, aus dem Blutkreislauf in die Niere geschleust wird, um dort aktiviert zu werden. Die Forschungsergebnisse geben möglicherweise auch einen Hinweis darauf, wie andere Steroidhormone im Körper an ihren Zielort gelangen, darunter auch solche, die das Wachstum bestimmter Brust- und Prostatatumoren fördern. Die mit 25.000 Mark dotierte Auszeichnung wurde ihm am 3. Mai 2000 in Wiesbaden überreicht.

Sofja Kovalevskaja-Preis für Michael Gotthardt

Der Mediziner Michael Gotthardt (32) ist einer von 29 jungen Forschern, die den neuen Sofja-Kovalevskaja-Preis der Alexander von Humboldt-Stiftung erhalten haben, um eine eigene Forschungsgruppe in Deutschland aufzubauen. Einige Jahre zuvor hatte er in der Gruppe von Prof. Michael Strauss am MDC gearbeitet, bevor er in die USA ging. Dort war er an den Universitäten in Dallas (Texas) und Pullman (Washington) tätig. Mit Hilfe des mit 1,2 Millionen Euro dotierten Stipendiums kehrte er 2002 für eine Förderperiode von 3 Jahren an das MDC zurück.

MDC und Forschungszentrum Karlsruhe erhalten ersten Technologietransferpreis des Bundesforschungsministeriums

Der mit insgesamt 400.000 Mark dotierte „Preis des Bundesministeriums für Bildung und Forschung für Gründungsinitiativen, vergeben durch die Karl Heinz Beckurts-Stiftung“ ist zu gleichen Teilen an das MDC und das Forschungszentrum Karlsruhe gegangen. Mit dem zum ersten Mal verliehenen Preis will das Bundesforschungsministerium (BMBF) Initiativen außeruniversitärer Forschungseinrichtungen bei der Entwicklung von Geschäftsideen und bei der Gründung technologieorientierter Unternehmen unterstützen. Der Preis wurde den beiden Mitgliedern der Helmholtz-Gemeinschaft Deutscher Forschungszentren (HGF) am 8. Dezember 2000 im Rahmen einer Festveranstaltung der Beckurts-Stiftung in München vom Parlamentarischen Staatssekretär im Bundesforschungsministerium, Wolf-Michael Catenhusen, überreicht. Das MDC erhält den Preis für den „Aufbau eines Netzwerkes von biomedizinischen Firmen und Forschungseinrichtungen bis hin zu Venture-Capital-Gesellschaften, aus dem eine große Zahl von Firmenansiedlungen resultiert“.

InnoRegio project launched in Berlin-Buch

In the InnoRegio competition announced by the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF) for regional development in the new German states, Berlin-Buch, represented by the Gesundheitsregion (Health Region) Berlin-Buch e.V., was one of the few winners of the second round. Almost 5 million Euro was granted by the BMBF to establish a regional network of competence in order to encourage the development of novel therapeutic approaches, biomedical technologies and clinical applications that will have a decisive impact on regional development. As a first step in achieving this aim, the network management was set up at the beginning of 2002 on the biomedical campus Berlin-Buch. The main task of the management team, consisting of a project manager and a project assistant, is to encourage and coordinate joint projects between science, biotechnology and clinical research. The key goal of InnoRegio and its management is to initiate a network of experts and technological platforms for the development and testing of new therapeutic agents, beginning with early studies of Human Genome Epidemiology, functional genomics, screening molecular targets for drugs, drug design, GMP-production of samples for clinical trials and finally testing new agents in Phase I–III clinical trials. All these activities involving local partners from diverse backgrounds will be supported by a company, which will be founded in the near future to provide site management of the activities of our partners in clinically oriented research and development. Projects concerned with education of study personnel and the improvement of the public understanding of genetics and clinical trials will complement the portfolio of InnoRegio.

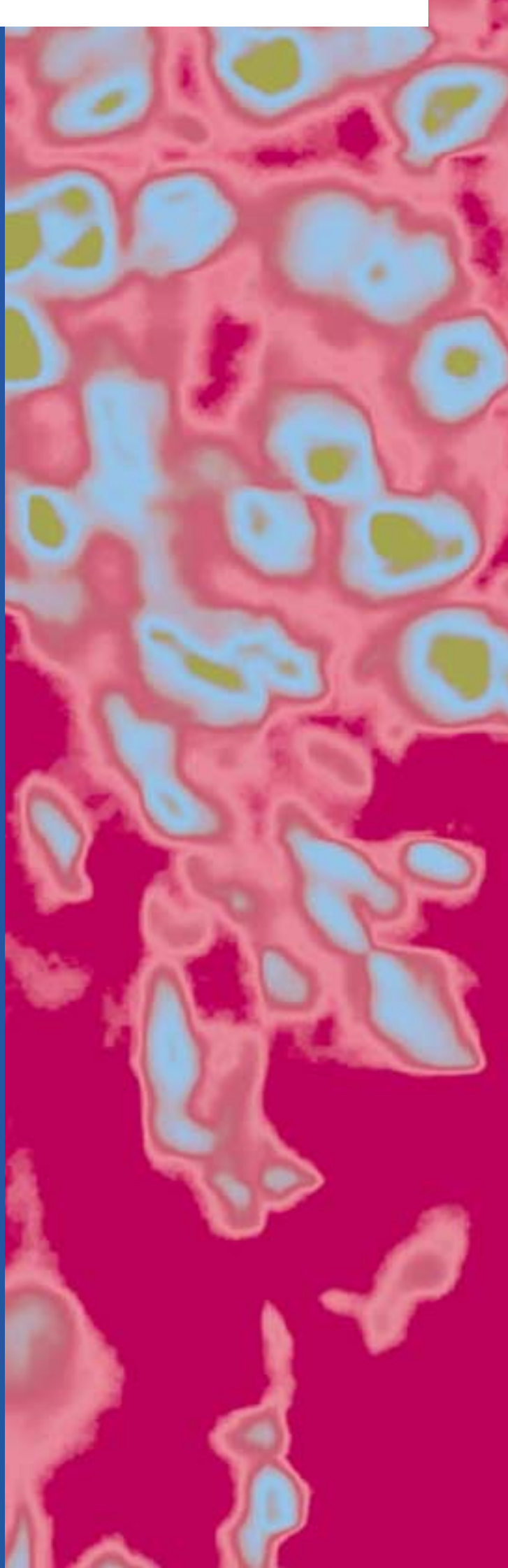
The first projects initiated in spring 2002 are focusing on the development of novel procedures for the screening of molecular drug targets and subsequent drug design as well as on the establishment of a new technological platform for gene assays to allow drug monitoring at a molecular level during clinical trials.

Ein InnoRegio Projekt für Berlin-Buch

Im InnoRegio Wettbewerb, den das BMBF zur Förderung der regionalen Entwicklung in den neuen Bundesländern ausgerufen hatte, gehörte Berlin-Buch zu den wenigen Gewinnern der zweiten Runde. Buch wurde repräsentiert durch die Gesundheitsregion Berlin-Buch e. V. Das BMBF stellte einen Betrag von nahezu 5 Millionen Euro zur Verfügung, um ein regionales Kompetenz-Netzwerk zu etablieren, mit dessen Hilfe die Entwicklung neuartiger therapeutischer Ansätze, biomedizinischer Technologien und klinischer Anwendungen erleichtert werden soll, die einen entscheidenden Einfluss auf die regionale Entwicklung ausüben können. Als ersten Schritt zur Realisierung des Programms wurde zu Beginn des Jahres 2002 auf dem biomedizinischen Campus Berlin-Buch das Netzwerk Management etabliert. Die Hauptaufgabe dieses Management, zu dem ein Projektmanager und eine Projektassistentin gehören, liegt in der Stimulierung und Koordinierung gemeinschaftlicher Projekte zwischen der Wissenschaft, der Biotechnologie und der klinischen Forschung. Das oberste Ziel der InnoRegio und des dazugehörigen Managements besteht darin, ein Netzwerk von Experten und technologischen Plattformen zu knüpfen, das der Entwicklung und Prüfung von neuen therapeutischen Agenzien dient, beginnend mit frühen Studien der Humangenom-Epidemiologie, der funktionellen Genomik, der Reihenuntersuchung von molekularen Angriffspunkten für Medikamente, dem Design von Medikamenten, der Produktion von Proben für klinische Prüfungen nach den GMP-Richtlinien bis hin zu der Prüfung neuer Agenzien in der Klinik von Phase I bis Phase III. Alle diese Aktivitäten von verschiedenen Partnern mit jeweils verschiedenem Hintergrund werden von einer Firma unterstützt, die in nächster Zukunft gegründet wird, um die verstreuten Aktivitäten der Partner in der klinisch orientierten Forschung und Entwicklung von einer Stelle aus zu managen. Projekte, die der Ausbildung des Prüfungspersonals und dem verbesserten „public understanding“ von Genetik und klinischen Prüfungen dienen, werden das Portfolio von InnoRegio ergänzen.

Die ersten Projekte, die im Frühjahr 2002 in die Wege geleitet wurden, konzentrieren sich sowohl auf die Entwicklung neuartiger Verfahren für die Reihenuntersuchung von molekularen Angriffspunkten für Medikamente mit anschließendem „drug design“ als auch auf die Errichtung einer neuen technologischen Basis für Gentests, die auch eine Ermittlung der Medikamente auf der molekularen Ebene während klinischer Prüfungen erlauben.

Genetics, Bioinformatics and Structural Biology



Genetics, Bioinformatics and Structural Biology

This Research Program combines groups that use approaches based on genetics, functional genomics, bioinformatics and structural biology to study the genetic and molecular basis of disease. Problems addressed in this context range from the genetic epidemiology of cardiovascular disease and cancer to developmental biology, signal transduction and macromolecular structure, function, and stability. Animal models are of particular importance for elucidating gene function during embryonic development and in the adult organism. Close collaboration with the Franz Volhard Clinic promotes research into the molecular basis of cardiovascular disease. Other research, carried out within the Genetics, Bioinformatics and Structural Biology Program, is directed at breast cancer. In-depth studies of the function of single genes and gene products in cells and tissues are complemented by systematic linkage analyses of genetic markers as carried out in the MDC Gene Mapping Center. Along with various functional genomics approaches, gene mapping and genetic analyses will be important in the planned MDC Center of Medical Genomics that is a logical consequence of the work currently carried out in this Research Program. Another research discipline of great importance to the new Center is bioinformatics, which already supports and connects the genetic, genomic and structure-oriented research in the department.

Carmen Birchmeier and colleagues are studying signal transduction processes that regulate cell migration and cell lineage determination during development. Using recently established mouse models with ubiquitous or conditional gene defects in the tyrosine kinase receptor ErbB2, its ligand neuregulin-1, and the transcription factor Sox10, they have identified signaling pathways controlling the migration of neural crest cells and their differentiation into the Schwann cell lineage. Furthermore, they identified crucial roles for the scatter factor/hepatocyte growth factor, its receptor c-Met, and the homeobox gene Lbx1 in the migration of myogenic precursors to the limb buds and in skeletal muscle formation.
The group of Thomas Willnow also employs mouse models and conditional mutagenesis to elucidate the role of novel gene products. The focus of these studies is the functional

Genetik, Bioinformatik und Strukturbiologie

Die in diesem Experimentellen Forschungsprogramm vereinten Gruppen untersuchen genetische und molekulare Grundlagen von Erkrankungen mit Ansätzen, die auf Genetik, funktioneller Genomforschung, Bioinformatik und Strukturbiologie beruhen. Die bearbeiteten Problemfelder reichen von der genetischen Epidemiologie kardiovaskulärer Erkrankungen und von Krebs zu Aspekten der Entwicklungsbiologie, zellulären Signalübertragung und makromolekularen Struktur, Funktion und Stabilität. Von besonderer Bedeutung für die Aufklärung genetischer Faktoren bei Embryonalentwicklung und im adulten Organismus sind Tiermodelle. In enger Kooperation mit der Franz-Volhard-Klinik der Charité werden molekulare Grundlagen kardiovaskulärer Erkrankungen erforscht. Daneben wird im Rahmen des Forschungsprogramms auch die Genetik von Brustkrebs untersucht. Vertiefte Untersuchungen der Funktion einzelner Gene oder Genprodukte in Zellen und Geweben werden im Genkartierungszentrum des MDCs durch systematische Analyse der Kopplung zwischen genetischen Markern ergänzt. So wie diverse Ansätze der funktionellen Genomforschung werden auch Genkartierungen und genetische Analysen für das geplante Zentrum für Medizinische Genomforschung des MDCs wichtig sein. Dieses Zentrum darf als logische Fortentwicklung der jetzt im Forschungsprogramm Genetik, Bioinformatik und Strukturbiologie durchgeführten Arbeiten angesehen werden. Die Forschungsdisziplin Bioinformatik wird für das neue Zentrum von ähnlich großer Bedeutung sein wie für das bestehende Forschungsprogramm, in dem sie genetische, genomische und strukturbiologische Forschung schon jetzt unterstützt und verbindet.

Carmen Birchmeier und ihre Kollegen studieren Signalübertragungsvorgänge, welche die Zellmigration und Zelltyp-Spezifizierung während der Embryonalentwicklung regulieren. Ausgehend von unlängst etablierten Maus-Modellen mit ubiquitären oder konditionalen Defekten in den Genen des Tyrosinkinase-Rezeptors ErbB2, seines Liganden Neuregulin-1 und des Transkriptionsfaktors Sox10 konnten Signalübertragungswege aufgezeigt werden, die die Migration von Neuralleistenzellen und ihre Differenzierung in Schwann-Zellen steuern. Darüber hinaus konnte die entscheidende Bedeutung des Pro-

characterization of the LDL receptor gene family, a class of multifunctional endocytic receptors. Recent work has uncovered an important role of megalin, a member of this gene family, in the cellular uptake and metabolism of the steroid hormone vitamin D. Megalin defects have been identified as a possible cause of vitamin D deficiency in humans.

Cardiovascular research depends heavily on model systems to elucidate genetic and non-genetic factors. Mouse, rat, and human disease models form the basis of a broad research spectrum targeted at unravelling critical pathways in cardiovascular pathology. Key molecules of the renin-angiotensin system and its counterplayer, the kallikrein-kinin system, have been modified in transgenic rodent models by Michael Bader's group to define organ-specific effects. In addition to endocrine systems, unrecognized genetic factors also appear to play a major role in systemic hypertension. Detlev Ganten and his team are addressing the issue of genetic factors by mapping quantitative trait loci in congenic rat strains. Tools such as transgenic technologies, linkage maps, genomic libraries, and expression chips, will provide the necessary resources for rat genomics, and not only for hypertension research. Genetic mapping information is often limited in the case of human diseases. However, with the identification of large families, twins, and other disease populations, several groups (Friedrich Luft, Arya Sharma, Jens Jordan, Maik Gollasch, Matthias Köhler, and Ludwig Thierfelder) have successfully used these resources to map and identify genetic factors relevant to human hypertension, obesity, metabolic and vascular diseases, as well as heart failure.

Genetic defects that predispose to the onset and progression of breast cancer in humans are the topics being addressed by Siegfried Scherneck and his coworkers. New studies include the detailed characterization of germ-line mutations in the breast cancer susceptibility genes BRCA1 and 2, and the fine mapping of novel tumor suppressor genes on chromosomes 6, 8 and 17.

The Gene Mapping Center of the MDC (Peter Nürnberg) provides techniques that permit the positional cloning of genes underlying genetic diseases. This facility provides high-throughput genetic linkage analysis with polymorphic microsatellite markers to identify the chromosomal location of candidate genes. Recently, gene mapping has been extended from the localization of genes underlying monogenic traits to identifying genetic factors involved in complex disease. The bioinformatics group of Jens Reich and Peer Bork is analyzing individual variations in the human genome and their role in lipid metabolism. By evaluating the importance of single nucleotide polymorphisms and splicing variants in the human genome, they are attempting to identify lipid traits as risk factors for arteriosclerosis.

Within the structural biology program of the MDC, three-dimensional structure analysis of proteins and nucleic acids by X-ray diffraction is a central theme of Udo Heinemann's research. His group has recently addressed problems of structure- and sequence-specific protein-nucleic-acid recognition, electron transfer in cytochrome P450 systems, the conformational stability of the ubiquitous cold shock domain, the function of LG domains in the sex-hormone binding globulin, and

teins Scatter Factor/Hepatozyten-Wachstumsfaktor, seines Rezeptors c-Met und des Homeobox-Gens Lbx1 für die Migration myogener Vorläufermoleküle zu den Extremitäten-Knospen und bei der Skelettmuskelbildung nachgewiesen werden.

Die Gruppe um Thomas Willnow setzt ebenfalls Maus-Modelle und konditionelle Mutagenese ein, um die Funktion neuer Genprodukte zu untersuchen. Der Schwerpunkt ihrer Untersuchungen liegt in der funktionellen Beschreibung der LDL-Rezeptor-Genfamilie, einer Klasse multifunktioneller Transportrezeptoren. Unlängst konnte eine wichtige Rolle des Megalins, eines Mitglieds dieser Genfamilie, in der zellulären Aufnahme und dem Metabolismus des Steroidhormons Vitamin D beschrieben werden. Megalindefekte wurden als mögliche Ursache des Vitamin-D-Mangels beim Menschen entdeckt.

Kardiovaskuläre Forschung bedarf zum Studium genetischer und nicht-genetischer Faktoren geeigneter Modellsysteme. Maus, Ratte und menschliche Erkrankungsmodelle bilden die Grundlage eines auf die Aufklärung kritischer Mechanismen kardiovaskulärer Pathologie abzielenden Forschungsspektrums. Schlüsselmoleküle des Renin-Angiotensin-Systems und seines Gegenparts, des Kallikrein-Kinin-Systems, wurden von Michael Baders Gruppe zur Beschreibung organspezifischer Effekte modifiziert. Zusätzlich zu endokrinen Systemen scheinen bei der Ausbildung des systemischen Bluthochdrucks noch unbekannte genetische Faktoren eine Rolle zu spielen. Detlev Ganten und seine Gruppe untersuchen genetische Faktoren kardiovaskulärer Erkrankungen durch Kartierung quantitativer Loci, die einen Phänotyp in congenen Rattenstämmen bestimmen. Analytische Werkzeuge wie transgene Techniken, genetische Kopplungs-analysen, genomische Bibliotheken und Expressions-Chips versprechen die bei der Genomanalyse der Ratte benötigten Ressourcen, nicht nur für die Erforschung des Bluthochdrucks, bereit zu stellen. Für Erkrankungen des Menschen liegen oft nur begrenzte Genkartierungsdaten vor. Mit der Identifizierung großer Familien, von Zwillingen und anderen krankheitsrelevanten Populationen haben einige Gruppen (Friedrich Luft, Arya Sharma, Jens Jordan, Maik Gollasch, Matthias Köhler und Ludwig Thierfelder) diese Ressourcen jedoch erfolgreich genutzt, um genetische Faktoren mit Bezug zu Bluthochdruck, Übergewicht, metabolischen und Gefäßerkrankungen, sowie Herzversagen beim Menschen zu identifizieren.

Genetische Anlagen für das Auftreten und Fortschreiten von Brustkrebs in der Bevölkerung werden von Siegfried Scherneck und seinen Mitarbeitern untersucht. Neuere Studien beschäftigten sich mit eingehenden Analysen von Keimbahnmutationen in den Brustkrebs-Genen BRCA1 und 2, sowie der Feinkartierung neuer Tumorsuppressorgene auf den Chromosomen 6, 8 und 17.

Das Genkartierungszentrum des MDCs (Peter Nürnberg) stellt Techniken zur Verfügung, die letztendlich die Positions-Klonierung krankheitsauslösender Gene erlaubt. Diese Einrichtung ist für hohen Durchsatz bei genetischen Kopplungsanalysen mit polymorphen Mikrosatellitenmarkern zur Identifizierung der chromosomalen Lage eines Kandidatengens ausgelegt. Die Anwendbarkeit der Genkartierung wurde in jüngster Zeit von der Genanalyse bei monogenen Defekten auf die Identifizierung genetischer Faktoren, die an komplexen

the recognition of cell surface receptors, such as tissue factor, by monoclonal antibodies. The group also plays a leading role in the Protein Structure Factory, a Berlin-based structural genomics project devoted to establishing a technical basis for high-throughput protein structure analysis.

Computer algorithms for the systematic conformational description of nucleic-acid helices and the treatment of electrostatics in simulations of nucleic-acid structure and ligand binding are being developed in the group led by Heinz Sklenar. These tools are being used to predict conformational properties of gene regulatory DNA sequences, non-canonical structural motifs in RNA, and singlet-oxygen generating dyes bound to DNA.

Protein misfolding and non-native protein conformations related to amyloidoses, diseases characterized by deposits of β-stranded protein aggregates in tissue, are in the focus of Gregor Damaschun's research. By combining several experimental techniques, his group is studying the folding pathways and kinetics of a number of polypeptides, including the Alzheimer Aβ peptides, to help understand the mechanisms of pathological protein misfolding.

The group of Christiane Jung is interested in the dynamic behavior of the thiolate heme proteins, cytochrome P450 and NO synthase, during redox reactions. Using time-resolved Fourier-transform infrared spectroscopy discrete reaction intermediates associated with electron transfer have been identified for cytochrome P450, and structural features of the active site of NO synthase have been characterized. Antibody-peptide interactions and initiator-tRNA binding by the bacterial initiation factor IF2 have been investigated in the laboratory of Heinz Welfle. These studies used spectroscopic (circular dichroism, fluorescence, infrared, and Raman), as well as calorimetric methods.

Carmen Birchmeier-Kohler, Udo Heinemann, Friedrich C. Luft, Jens G. Reich, Ludwig Thierfelder, Thomas Willnow

Erkrankungen beteiligt sind, erweitert. Die Bioinformatik-Gruppe von Jens Reich und Peer Bork beschäftigt sich mit der Analyse individueller genetischer Variationen beim Menschen und ihrer Rolle im Fettstoffwechsel. Durch Untersuchung der Bedeutung von Einzel-Nukleotid-Polymorphismen und Spleißvarianten im Humangenom wird versucht, Fettstoffwechsel-Anlagen als Risikofaktoren für Arteriosklerose zu identifizieren.

Innerhalb der Strukturbiologie am MDC beschäftigt sich Udo Heinemanns Gruppe mit der Analyse dreidimensionaler Strukturen von Proteinen und Nukleinsäuren durch Röntgenbeugungsmethoden. In jüngerer Zeit hat sie Aspekte der struktur- und sequenzspezifischen Protein-Nukleinsäure-Erkennung, des Elektronentransfers im Cytochrom P450-System, der konformationellen Stabilität der ubiquitären Kälteschockdomäne, der Funktion von LG-Domänen, wie sie im Sexualhormonbindenden Globulin vorkommen, sowie der Erkennung von Zelloberflächen-Rezeptoren vom Typ des Gewebsfaktors durch monoklonale Antikörper untersucht. Darüber hinaus spielt die Gruppe eine führende Rolle in der „Proteinstrukturfabrik", einem Berliner Gemeinschaftsprojekt der strukturellen Genomforschung mit dem Ziel der Etablierung einer technischen Infrastruktur für die Proteinstrukturanalyse bei hohem Durchsatz.

Computeralgorithmen für die systematische Konformationsanalyse von Nukleinsäurehelices und die Behandlung der Elektrostatik in Simulationen der Nukleinsäurestruktur und der Ligandenbindung werden in Heinz Sklenars Gruppe entwickelt. Diese Werkzeuge dienen der Vorhersage von Konformationseigenschaften genregulatorischer DNA-Sequenzen, nicht-kanonischer Strukturmotive in RNA und der Bindung von Singulett-Sauerstoff freisetzenden Farbstoffen an DNA.

Die Fehlfaltung von Proteinen und nicht-native Proteinkonformationen mit Bezug zu Amyloiderkrankungen, die durch die Ablagerung β-strukturierter Proteinaggregate in Gewebe charakterisiert sind, stehen im Mittelpunkt der Arbeit bei Gregor Damaschun. Durch Kombination verschiedener experimenteller Techniken untersucht seine Gruppe Faltungswege und -kinetik einer Reihe von Polypeptiden, darunter Alzheimer Aβ-Peptide, um Mechanismen pathologischer Proteinfehlfaltungen verstehen zu helfen.

Christiane Jungs Gruppe interessiert sich für das dynamische Verhalten der Thiolat-Haem-Proteine Cytochrom P450 und NO-Synthase während Redoxreaktionen. Unter Anwendung zeitaufgelöster Fourier-Transform-Infrarotspektroskopie wurden diskrete Reaktionsintermediate beim Elektronentransfer bei Cytochrom P450, sowie strukturelle Eigenschaften des aktiven Zentrums der NO-Synthase beschrieben. Wechselwirkungen zwischen Antikörpern und Peptiden, sowie zwischen Initiator-tRNA und dem bakteriellen Initiationsfaktor IF2 wurden im Labor von Heinz Welfle untersucht. Dabei kamen spektroskopische (Circulardichroismus, Fluoreszenz, Infrarot und Raman) und kalorimetrische Methoden zum Einsatz.

Carmen Birchmeier-Kohler, Udo Heinemann, Friedrich C. Luft, Jens G. Reich, Ludwig Thierfelder, Thomas Willnow

Molecular Biology and Genetics of Cardiovascular Disease

Detlev Ganten
Norbert Hübner

Analysis of complex cardiovascular diseases 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 systematic genetic investigation of the rat is substantial.

Over the past years we have demonstrated that multiple chromosomal loci in rat models contribute to blood pressure regulation and hypertension. Independent of 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 are causative for different cardiovascular disease phenotypes.

To localize the disease genes within chromosomal regions linked to quantitative traits (e.g. blood pressure), we are establishing multiple congenic rat strains. These congenic strains are being developed by introgressing disease alleles encompassing the quantitative trait locus (QTL) into non-affected reference strains 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 allow the successive fine mapping within a QTL.

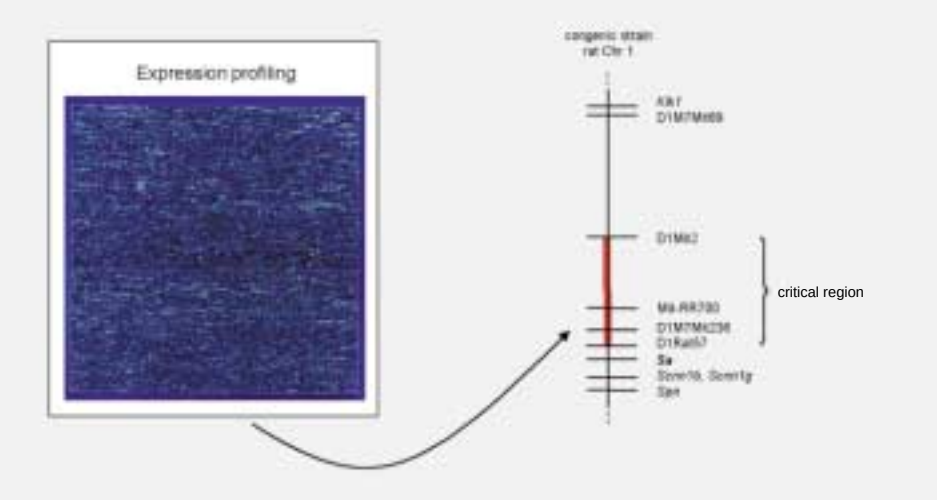
Production and high-throughput characterization of genomic resources for the rat genome

The ultimate identification of disease-relevant genes within QTLs by positional cloning requires the availability of a variety of genomic tools, such as large-insert genomic library clones, cDNA libraries and mapping resources. As a partner of national and international rat genome research efforts our group has produced various tools for the rat genome, among them the first rat YAC library, a high-resolution mapping cross, and a hybridization based Interspersed Repetitive Sequence (IRS-)PCR marker system. A set of about 800 IRS markers has been assigned to the rat genetic and radiation hybrid (RH) maps. A preliminary physical framework map has been produced based on hybridization data from this set of markers against high density gridded filters representing about 90,000 YAC clones (corresponding to 20-fold coverage) of the rat genome.

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 arrays of cDNA clones or gene-specific oligonucleotides 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 (see Figure).

Transgenic rat technology

In order to study the functional relevance of genes linked to hypertension and stroke, transgenic rats have been 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 have helped to reveal the physiological functions of local renin-angiotensin systems in tissues. Furthermore, transgenic rats carrying the human renin and angiotensinogen genes are excellent models to study pregnancy-induced hypertension and hypertension-induced end-organ damage, particularly in the kidney. In addition, numerous other transgenic rat models for the study of cardiovascular physiology have been generated and analyzed in collaboration with other groups. Furthermore, transgenic technology in the rat is being developed further by the generation of transgenic animals with large genomic constructs and the establishment of knockout technology for this species. Major efforts are directed towards the establishment of ES cells in rats.



Schematic representation of a congenic rat strain and a gene chip analysis. The transcriptional level of several thousand genes can be investigated simultaneously. Genes presenting evidence for allele specific transcriptional regulation are mapped to the rat genome. If they map to the identified critical region they represent a positional candidate gene.

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Molecular Biology of Peptide Hormones

Michael Bader

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

Renin-angiotensin system

The renin-angiotensin system (RAS) is central to blood pressure regulation and, therefore, has been studied in detail by transgenic techniques.

A major focus of our research is the tissue RAS in the brain. Transgenic rats expressing an antisense RNA against angiotensinogen exclusively in brain astrocytes have been developed and show a decreased local concentration of this protein and lowered blood pressure and plasma vasopressin levels. These animals are suitable models for the study of the function of local angiotensin production in the brain. Using these rats, we showed that central angiotensin modulates circadian rhythms and the baroreceptor reflex. Furthermore, it is involved in the hypertensive and hypertrophic effects of circulating angiotensin.

The role of the RAS in hypertension-induced end-organ damage is of major clinical importance. In a novel transgenic mouse model, we studied the function of locally produced angiotensin in the development of cardiac hypertrophy and nephrosclerosis. These mice carried a targeted disruption of the angiotensinogen gene which was compensated by a rat transgene exclusively expressed in liver and brain, but not in kidney and heart, where the angiotensinogen gene is expressed in normal mice. Because of elevated plasma angiotensinogen levels, the animals are hypertensive but they suffer less damage to the target organs as a result of the lack of local angiotensin synthesis.

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 developed expressing the human tissue kallikrein gene under the control of the heavy-metal responsive metallothionein promoter. The animals express the transgene in all organs tested and excrete human tissue kallikrein in the urine. In these rats, blood pressure and its diurnal rhythm, as measured by telemetry, is significantly reduced compared with control rats. The hearts of the animals are protected against ischemic and hypertrophic injury.

The functions of the kinin B1 receptor are an enigma. To investigate them, we developed mice lacking this subtype. The resulting animals exhibited analgesia and altered inflammatory reactions demonstrating the important role of the B1 receptor in pain transmission and inflammation.

Embryonic stem cell technology

Using embryonic stem cell technology, the gene for the smooth muscle myosin heavy chain has been deleted by homologous recombination. Lack of this gene still allowed smooth muscle contraction indicating redundancy of the contractile proteins. Furthermore, mice lacking tryptophan hydroxylase were developed and the genotype of these serotonin-deficient animals is under investigation.

In order to also allow gene-targeting experiments in the rat, which is more suitable for research on cardiovascular diseases than the mouse, we are establishing embryonic stem cells and cloning technology for this species.

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Genetics, Etiology, and Pathogenesis of Hypertension, Vascular Injury, and Renal Diseases

Friedrich C. Luft

Summary

This group is interested in the genetics and pathogenesis of hypertension, vascular, and renal diseases. Sylvia Bähring leads a team concentrating on the molecular genetics of blood pressure regulation and lipid metabolism. Here, the aim is to find important regulatory genes by means of linkage, association, and positional cloning. Volkmar Gross focuses on sophisticated cardiovascular physiology in gene-targeted mice. He combines this technology with gene expression arrays. Dominik N. Müller concentrates his attention on the vascular wall. His team uses unique, transgenic rodent models to elucidate mechanisms responsible for hypertension-induced end-organ damage. Ralf Dechend is pursuing preeclampsia research in patients and in animal models. Ralf Kettritz is studying human vasculitis while Marek Drab has succeeded in disrupting the gene for caveolin-1.

Molecular genetics

Sylvia Bähring has been pursuing the gene responsible for autosomal-hypertension and brachydactyly. She, Hakan Toka, and Friedrich C. Luft have studied families from Turkey, France, Canada, and the United States with this problem. Affected persons have severe hypertension and die of stroke before the age of 50. The gene is mapped on chromosome 12p and attempts are being made to find the gene by positional cloning. Hussam Al-Kateb is working on autosomal-recessive hypercholesterolemia. His family has a mutation in the gene coding for an LDL receptor adapter protein on chromosome 1. Interestingly, the family also shows linkage to a site on chromosome 13q that the team has previously identified as the site of a putative “lipid-lowering” gene. Andreas Busjahn has focused on monozygotic and dizygotic twins. He and his associates recently identified the PPARγ gene locus as the site of a gene involved in the process of dizygotic twinning.

Vascular research

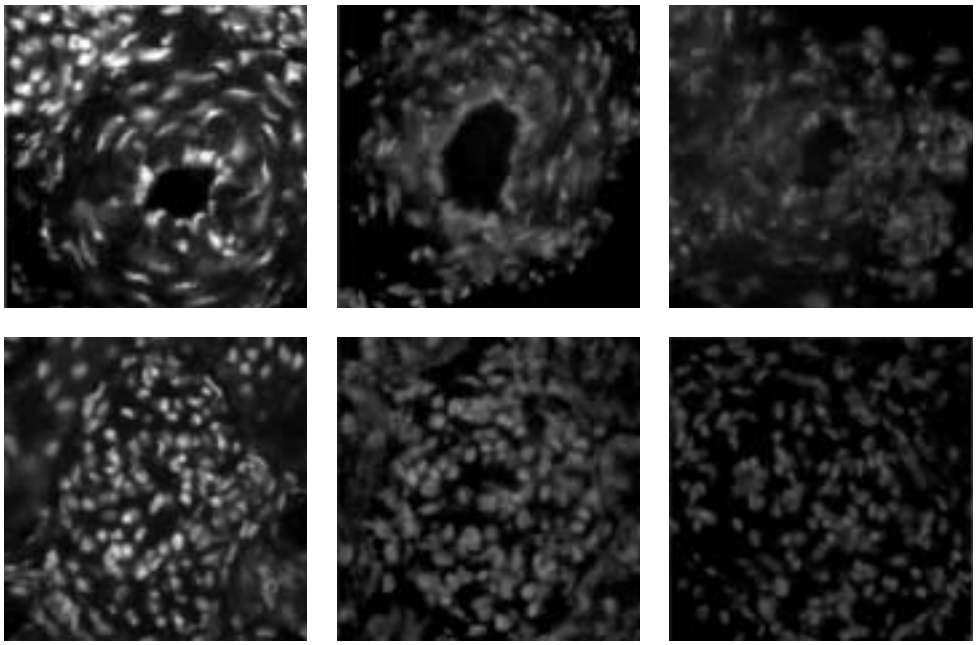
Volkmar Gross has succeeded in establishing blood pressure telemetry, cardiac catheterization, and sophisticated methods of renal function monitoring in gene disrupted mice. He has characterized mice lacking the angiotensin AT2 receptor and other mice lacking the bradykinin BK2 receptor. Other novel models are currently being studied. Coupling detailed physiology with microarray analyses promises to identify novel genes involved in cardiovascular disease. Dominik N. Müller has focused on angiotensin II-induced vascular damage. Reactive oxygen species generation, and activation of associated transcription factors, such as NF-κB and AP-1, are pivotal to this process. In collaboration with Claus Scheidereit, Müller has shown that aspirin treatment and other novel anti-inflammatory approaches can reduce vascular damage. A unique role for angiotensin II-induced adaptive immunity has been recently identified by the group.

Ralf Dechend, a young cardiologist from the department of Rainer Dietz, has become interested in preeclampsia. The team had previously shown that women with preeclampsia develop autoantibodies capable of stimulating the angiotensin II AT1 receptor. Dechend has pursued this issue further and has shown that NADPH oxidase is activated by these antibodies in human placental tissue. Ralph Kettritz is an authority on vasculitis, particularly on Wegener’s granulomatosis and he recently observed that anticytoplasmic antibodies signal neutrophils via the MAP kinase pathway. The process has an important bearing on neutrophil apoptosis.

Finally, Marek Drab spent four years in the laboratory of Teymuras Kurzchalia at the MDC where they succeeded in disrupting the caveolin-1 gene. The absence of caveolae in the mice impaired nitric oxide and calcium signaling in the cardiovascular system causing aberrations in endothelium-dependent relaxation, contractility, and maintenance of myogenic tone. In addition, the lungs of knockout animals displayed thickening of the alvaeolar septa caused by uncontrolled endothelial cell proliferation and fibrosis, resulting in severe physical limitations in caveolin-1-disrupted mice. The mice should prove invaluable for clarifying the importance of caveolae.

Milestones

Hermann Haller left the Nephrology Hypertension Department at the Franz Volhard Clinic to assume chairmanship of the Nephrology Department at the University of Hannover Medical School in Hannover, Germany. His position has been filled by Arya M. Sharma, whose research report is in this volume. Herbert Schuster founded the biotech company, INFOGEN, which specializes in genetic evaluations and risk assessment. He has been joined by Andreas Busjahn and Hans Knoblauch and close collaborations with the MDC have been maintained by these investigators. Ralf Dechend was awarded the Jan Brod prize (2000) for his preeclampsia work and Friedrich C. Luft was awarded the Arthur Corcoran Award of the American Heart Association (2000) and the Lingen Prize (2001) of the Lingen Foundation.



Hydroxy-ethidium method for determining reactive oxygen free radical production within the vascular wall (upper) and glomeruli (lower). Untreated double transgenic rats (dTGR) produce high amounts via local Ang II. Following dexamethasone treatment, this production is reduced to Sprague-Dawley (SD) levels.

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Gene Mapping and Identification in Monogenic and Complex Diseases

Peter Nürnberg

Positional cloning is now widely used for the identification of gene defects that are the basis of 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 allow 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 etiology of complex 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 a whole genome scan.
The Gene Mapping Center is a specialized laboratory for such high-throughput genotyping for gene mapping in monogenic as well as complex diseases. We have developed various sets of well established markers from the Généthon and the CHLC genetic maps with different marker densities to accommodate the requirements of special study designs. The laboratory is mainly funded by grants from the German Federal Ministry of Education and Research (BMBF). Since January 1997, we have participated in the German Human Genome Project and, since 2001, in the National Genome Research Network as one of the major core facilities. The laboratory also undertakes mapping projects for researchers from Germany and abroad.

Mapping of complex diseases

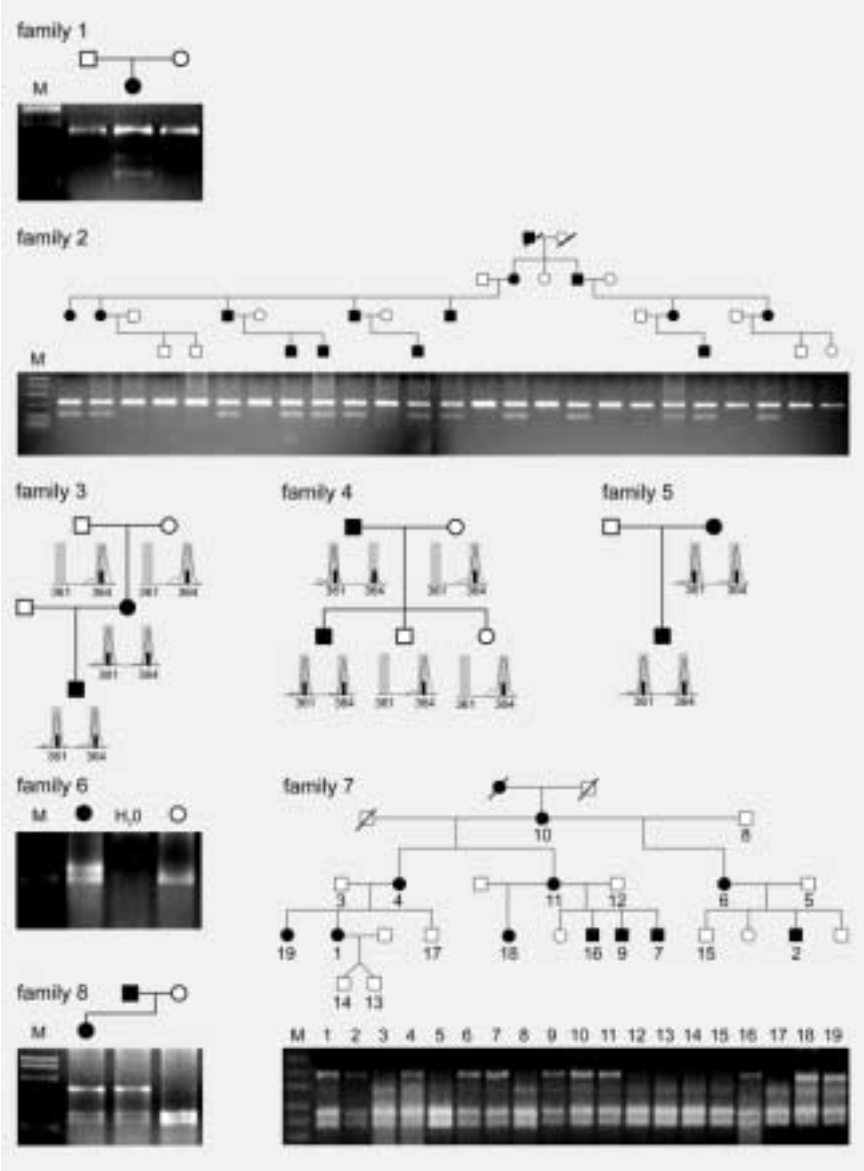
The main focus of the Gene Mapping Center is mapping of genetic factors in multifactorial diseases. This type of study involves the analysis of large numbers of phenotypically well characterized families. Hundreds of markers are used for genotyping and sophisticated biostatistical analyses are subsequently required to identify genetic loci that contribute to a complex disease. For this purpose we have established the necessary techniques and, particularly, automation of the experimental procedures. Our annual capacity is about 2,000,000 high-quality genotypes and we are working towards doubling this capacity by the end of the year. Currently, mapping is based upon highly informative microsatellite markers but, in the future, analysis will shift more towards single-nucleotide polymorphisms (SNPs). Five scientists are involved in project management, genotyping and technology development while 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 are also involved in data analysis.

A total of seven genome scans for complex diseases have been completed and two further studies are underway. In a European collaborative study of the genetics of atopic dermatitis, 199 families with two or more affected siblings each, totalling 839 probands, have been investigated and a major susceptibility locus identified on chromosome 3q21. In a second European collaborative study, 130 families comprising 617 probands have been investigated to identify genetic factors involved in susceptibility to common idiopathic generalized epilepsies (IGE). The linkage results provided significant evidence for a novel IGE susceptibility locus on chromosome 3q26 and suggestive evidence for two IGE loci on chromosomes 14q23 and 2q36.1. The susceptibility loci of both diseases are currently being pursued further with refined mapping and testing of positional candidate genes. We expect to gain important insights into the etiology of both disease groups.

Ongoing studies include an affected sib-pair study of type 2 diabetes in about 400 affected sib pairs from Saxony (PI Dr. Tom Lindner, University Hospital Würzburg) and genotyping of a study of the genetic factors in hypertension in collaboration with the Franz-Volhard Clinic on the MDC campus (Prof. Friedrich Luft). The design of the latter study is based on isolated populations and takes advantage of the restricted genetic heterogeneity in these populations.

Mapping of monogenic diseases

In contrast to complex diseases, mapping of monogenic traits requires less genotyping. Usually, it is sufficient to analyze 30 probands or less. The statistical evaluation is different and often requires skilled interpretation, e.g. haplotyping. In the six years the laboratory has been in existence, about 50 monogenic traits have been mapped in humans. The underlying gene defect has been identified for several of these, completing the process of positional cloning. For instance, an autosomal dominant bone dysplasia, craniometaphyseal dysplasia (CMD), was found to be caused by heterozygous mutations in ANKH, the human ortholog of the mouse progressive ankylosis gene. The gene product is a multipass transmembrane protein and seems to form a channel for inorganic pyrophosphate (PPi). These data are important since they link the PPi pathway with bone formation and remodelling. Further analysis of the molecular pathology of CMD and related conditions is in progress. 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.



Cosegregation of *ANKH* mutations with the CMD phenotype. Eight families with mutations in *ANKH* are shown. In family 6, no DNA samples from the unaffected parents were available for analysis. Presence or absence of *ANKH* mutations previously identified by genomic sequencing is demonstrated by restriction analysis (families 1, 2, 6-8) or fragment size determination of PCR products (families 3-5). Filled square, affected male; filled circle, affected female; open square, normal male; open circle, normal female; /, deceased; M, size marker.

Another focus of the group is the molecular characterization of hereditary skin diseases. Congenital ichthyosis is a severe genodermatosis characterized by scaling of the skin over the entire body. Autosomal recessive congenital ichthyosis is both clinically and genetically heterogeneous. A new locus for this disorder has been identified on chromosome 17p by combination of linkage analysis and homozygosity mapping in families from Germany and Turkey.

The etiology of chronic pancreatitis, a continuing or relapsing inflammatory disease of the pancreas, is still unclear in many cases. Mutations in the cationic trypsinogen gene were only found in 11 of 96 unrelated cases analyzed. However, we have identified a strong association between chronic pancreatitis and mutations in the pancreatic secretory trypsin inhibitor gene (SPINK1) in this group.

In addition, we have also initiated mapping of monogenic traits in animal models, mainly the mouse. Several spontaneous and ENU-induced mutants have been mapped and, in six cases, the underlying mutations have been identified. The majority of the projects originated in external laboratories and clinics in Germany as well as England, France, The Netherlands, Canada, the Republic of South Africa, the United Arab Emirates, Australia, and other countries.

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Cardiovascular Molecular Genetics

Ludwig Thierfelder

Animal models for familial hypertrophic cardiomyopathy disease genes

All disease genes for autosomal dominant familial hypertrophic cardiomyopathy (FHC) code for proteins of the contractile machinery of striated muscle. This led to the definition that FHC is a disease of the sarcomere. In order to better understand functional and molecular disease pathways in FHC caused by alpha-tropomyosin mutations, we overexpress two alpha-tropomyosin mutations (Asp175Asn; Glu180Gly) in a transgenic rat model. The phenotype differs for each mutation: mutation Glu180Gly is functionally silent, whereas mutation Asp175Asn shows decreased Ca++-sensitivity of cardiac myofilaments. Although no gross cardiac hypertrophy is observed, animals carrying either mutation develop myocyte disarray and reexpress fetal cardiac genes as early molecular markers of cardiac hypertrophy. This model is suited to the investigation of early functional and molecular events in the pathogenesis of alpha-tropomyosin mutations. Because the physiology of cardiac myosin binding protein C (MybP-C; another FHC disease gene), especially its N-terminus, is not well known, we have developed a knock-in mouse model to study functional aspects of mutated cardiac MybP-C.

Molecular genetics of familial dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is a genetic disease caused by autosomal dominant mutations in 20-30% of cases. Only a few DCM disease genes are known and these belong to different classes of molecules (dystrophin; lamin A/C; beta myosin heavy chain, actin; desmin). In two large DCM families, positional cloning efforts in our laboratory have very 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 up to 114kb cDNA. 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 the heart is clinically affected.

Molecular genetics of arrhythmogenic right ventricular cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is cardiac condition and is 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 ~400 individuals at 50% risk of inheriting the condition. ARVC in these individuals is associated with a distinct electrocardiographic pattern and a life expectancy of <40y in males. Mutational analyses of approximately 15 genes located in a 2Mbp region on chromosome 3p25 should identify the responsible mutation.

Genetic studies of 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 not known whether INVC can be caused by germline mutations. Because a mutation of the G4.5 gene (Barth disease gene) has been found in a patient with infantile INVC, the G4.5 gene in over 15 adult INVC patients has been screened but no mutation has been found. We have further evaluated the pedigrees of two INVC index patients and identified several members also affected by INVC. This shows that INVC in the adult can be transmitted genetically. Additional candidate genes of the endomyocardial morphogenesis pathways are currently being screened for INVC-causing mutations.

Molecular genetics of pseudoxanthoma elasticum (PXE)

Pseudoxanthoma elasticum is an inherited systemic disorder of the elastic tissue characterized by degenerative calcification with subsequent disintegration and destruction of the elastic tissue of several organs. Cardiovascular disease encompasses a wide clinical spectrum from mental fatigue syndrome to early cardiovascular death due to myocardial infarction or, very rarely, gastrointestinal hemorrhage. We have mapped the PXE locus to a 500 kb interval on chromosome 16p13.1. and shown that mutations in a transmembrane transporter protein, ABC-C6 (also known as MRP-6), cause PXE.

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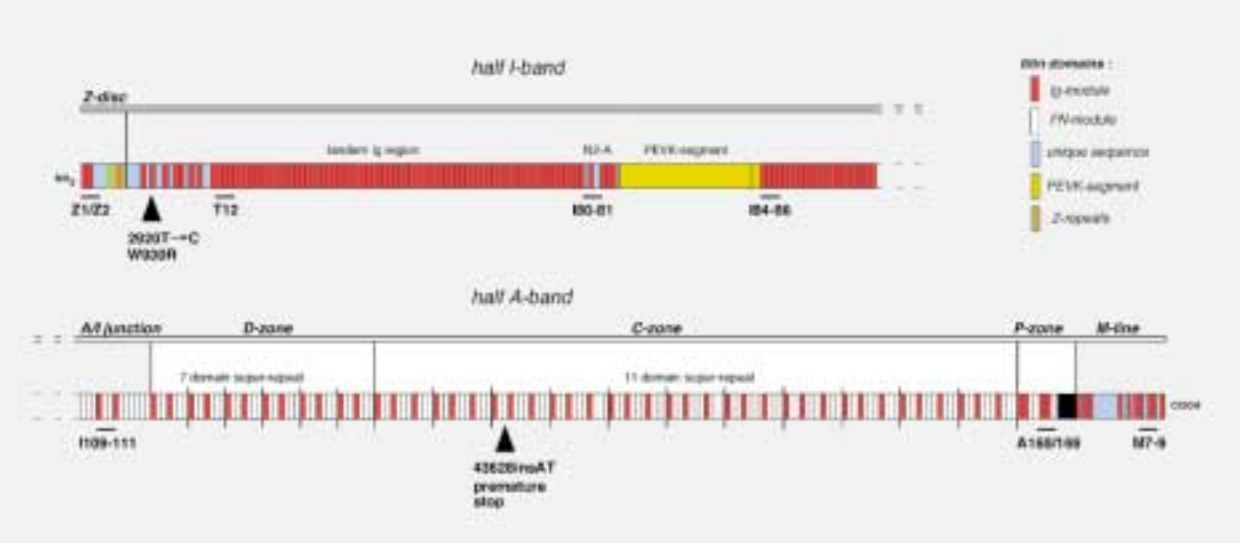

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Obesity and Hypertension

Arya M. Sharma

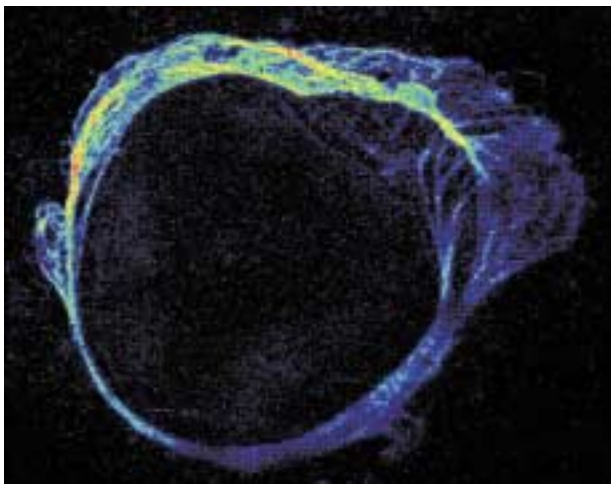
Recent years have seen a world-wide increase in the prevalence of overweight and obesity, affecting more than 30-40% of the populations in many countries. Obesity has long been recognized as one of the main risk factors for hypertension, but the molecular mechanisms underlying this relationship have yet to be understood. Recent studies have shown that adipose tissue is an important source of a host of factors that can directly contribute to cardiovascular function. Furthermore, recent developments have led to the identification of a variety of pharmacological targets for obesity therapy. The main research focus of this group is a study of the importance of adipose tissue for cardiovascular regulation and to address the cardiovascular effects and efficacy of antiobesity medications.

Expression of "cardiovascular" genes in human adipose tissue

Adipose tissue is now recognized to express a variety of genes with well-known cardiovascular function. These include angiotensinogen, endothelin, nitric oxide, and leptin. The function of these genes in adipose tissue is not clear. In order to explore the expression pattern of cardiovascular and other genes in human adiposes tissue and their relationship to obesity, hypertension and the metabolic syndrome, we are performing adipose-tissue biopsies in a large number of clinically well-characterized obese and non-obese patients with and without hypertension. Gene-expression profiles are being examined using a glass – slide-based microarray technique. These studies are funded by the Deutsche Human-Genomprojekt and should provide important insights into the molecular mechanisms related to obesity and hypertension.

Role of the renin-angiotensin system in adipocyte growth and differentiation

We have recently reported that all essential components of the renin-angiotensin system are widely expressed in human adipose tissue (Figure). We have now shown that this system is intimately involved in the adipogenic differentiation of human preadipocytes and also that the activity of this system is markedly elevated in patients with obesity, hypertension and insulin resistance. We hypothesize that increased activity of this system accounts for failure of adipocyte differentiation and may, therefore, contribute to the development of type 2 diabetes and/or hypertension in obese individuals.



Expression of AT1 receptors on a human fat cell
(Confocal microscopy: Gorzelniak/Quass)

Role of perivascular adipose tissue in vascular regulation

Most blood vessels are intimately surrounded by substantial amounts of adipose tissue. As adipose tissue is now recognized as a source of a variety of vasoactive substances, we examined the potential role of perivascular adipose tissue in the regulation of arterial function. We found that perivascular adipose tissue markedly attenuated the contractile response to vasoactive substances (Ang II, serotonin, phenylephrine), an effect that appears to be mediated by one or more factors that inhibit the ATP-dependent K-channel in vascular smooth muscle cells and are not dependent on NO production. Based on these findings, we propose that adventitial adipose tissue is an important regulator of vascular tone and may serve as an important interface between tissue demand and blood flow. Following two decades of intensive research on endothelial dysfunction, we believe that it is now time to consider the role of "adventitial dysfunction" in vascular disease.

Genetics of obesity and hypertension

Both obesity and hypertension are complex disorders influenced both by environmental as well as genetic factors. Several genes have recently been identified in rare monogenic

forms of obesity and hypertension, but the importance of these genes for the development of non monogenic forms of hypertension remains to be explored. We are currently conducting several studies using both a population-based cross-sectional as well as a family-based approach in an attempt to identify genetic variants that can account for the development of these disorders.

Pharmacological management of obesity hypertension

Although obesity accounts for a considerable proportion of cardiovascular morbidity and mortality, the efficacy and outcome of cardiovascular therapies have not been extensively studied in obese patients. Thus, whereas for non-obese patients the utility and efficacy of pharmacological intervention has been well established, these principles need to be reassessed regarding their utility and efficacy in obese patients. This is important, since obesity can affect both the pharmacokinetics and pharmacodynamics of various cardiovascular drug treatments. Furthermore, recent antiobesity drugs have been found to have a significant impact on cardiovascular regulation, but their role in the management of obesity hypertension remains to be defined. We are currently conducting several studies addressing these issues

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Disorders of the Autonomic Nervous System

Jens Jordan (Helmholtz fellow)

The main interest of the group is basic (mechanism-oriented) patient-oriented research in the field of clinical autonomic disorders, arterial hypertension, obesity, and genetics. One intention of our group is to combine patient-oriented research with basic science and genetics in the field of cardiovascular diseases. The purpose of our research is to develop new treatment strategies for patients with orthostatic hypotension, orthostatic intolerance, multiple system atrophy, pure autonomic failure, neurally-mediated syncope, and neurogenic hypertension based on a better understanding of the pathophysiology of these clinical syndromes. To elucidate the potential influence of candidate genes we participate in twin studies.

Genetic influences on cardiovascular regulation in health and disease

In a patient with orthostatic intolerance and her identical twin, we conducted extensive tests of autonomic nervous system regulation. We found evidence for impaired norepinephrine uptake mechanisms. Genetic testing revealed a functional mutation of the norepinephrine transporter gene causing the syndrome. We investigated blood pressure regulation in patients with monogenic hypertension, brachydactyly, and neurovascular brainstem contact. We found no difference in sympathetic nerve traffic or activation. However, blood pressure increases to phenylephrine were markedly augmented, compared with controls. Complete ganglionic blockade largely attenuated this difference, suggesting marked impairment of baroreflex buffering in these patients. In healthy dizygotic and monozygotic twins, we tested the hypothesis that baroreflex function, which has a central role in blood pressure regulation, is influenced by genetic factors. A large part of the variability in baroreflex function could be explained by genetic influences. In a subsequent association study, we found that part of the genetic influence on baroreflex function may be explained by a gene encoding a calcium-activated potassium channel.

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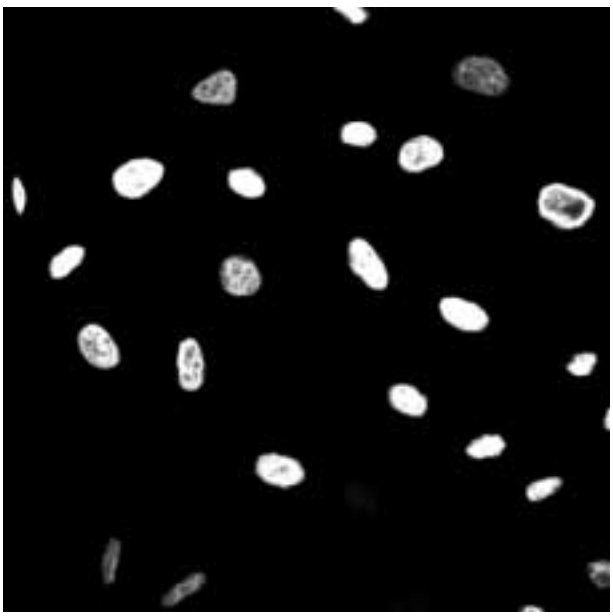
Characterization of newly identified human importin α proteins

Matthias Köhler (Helmholtz fellow)

Matthias Köhler and his group are working on nuclear protein import. In collaboration with Enno Hartmann and Dirk Görlich, he has identified four novel human importin α isoforms and demonstrated that they function as nuclear import factors. Using an in vitro import assay, he has shown that the α importins differ in their substrate specific import activity. He and his colleagues are trying to further characterize the function of the six known α importins in vitro and in vivo. Together with Jacqueline Franke, who is a doctoral fellow in his laboratory, he has recently demonstrated that nuclear import of an adenoviral E1A-NLS-BSA fusion protein is preferably mediated via importin α3 in vitro. Jacqueline Franke has been focusing on the analysis of the mechanisms mediating the nuclear import of nascent polypetide-associated complex (NAC). In collaboration with Brigitte Wiedmann, she has shown that non-ribosome-associated yeast NAC subunits can indeed translocate into the nucleus in vivo. Using in vitro assays and in vivo experiments, she has demonstrated that this transport is specific and can be mediated by several of the known import factors. Jacqueline Franke is now trying to find new import substrates via GST-pulldown assays using immobilized importin α proteins. Christina Quensel recently started to work as a postdoc in this group and she is trying to establish a model for inhibition of distinct α importins in cultured cells. The aim of this project is to test the hypothesis that the importin α proteins play a functional role during cellular differentiation and proliferation. Since the α importins are differentially expressed in various tissues, the group is also investigating if they are differentially regulated in chronic diseases. Together with Hermann Haller's group in Hannover and Eero Mervaala in Helsinki, Matthias Köhler and his team recently found that the newly identified importin α7 is strongly upregulated in two different rat models of diabetic nephropathy. This finding supports a role for importin α proteins during the development of this common renal disease.

Nuclear import of adenoviral E1A-NLS-BSA fusion proteins is mediated via importin α3 in vitro.



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Tumor Genetics

Siegfried Scherneck

The research program of this group is aimed at obtaining a better understanding of the genetic basis of cancers, particularly human breast cancers (BC). BC, one of the most common cancers affecting women, has been demonstrated to arise through a multi-step process in which a number of oncogenes and tumor suppressor genes (TSG) contribute to the cancer when their function is altered. Although about 90% of BC are sporadic, the remaining cases are heritable and caused by mutations of at least two TSG, BRCA1 and BRCA2. It is reasonably likely that at least one more highly penetrant susceptibility gene 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, identification and characterization of a number of genes that are somatically altered in sporadic BC as well as subsequent analysis of respective alterations in the germline of individuals of BC families (predisposing genes) will lead to a better understanding of the development and progression of BC. Advances in these areas may provide information about the clinical uses of these genes for predisposition testing, early detection, prognostication, and therapy selection.

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. Sümnich, H. Zeidler, K. Krause, the “Berlin Center for Hereditary Breast and Ovarian Cancer” in cooperation with the “German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC)” and M. Schwab (DKFZ, Heidelberg)

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 Krebshilfe”, we have participated in a comprehensive study to analyze the entire coding sequence of the BRCA1 and BRCA2 genes in about 1000 patients from German breast/ovarian cancer families. A total of about 80 BRCA1 and about 70 BRCA2 distinct deleterious mutants in about 300 patients 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 by extended control studies and investigation of the corresponding tumors. The identification of BRCA1 and BRCA2 genes has led to changes in the management and treatment of high-risk women.

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

B. Jandrig, S. Seitz, I. Lapidous, M. Plaumann, A. Schwartz, K. Wenzel, C. Zeller, R. Frege, K. Poppe, S. Werner, in cooperation with A. Meindl (Munich), N. Arnold (Kiel), D. Niederacher (Düsseldorf), K. Schmutzler (Bonn), B.M. Jockusch (Braunschweig), I. Petersen (Berlin), B. Hinzmann, A. Rosenthal (Berlin)

Over the last few years, the increasing resolution of genetic and physical maps has allowed the identification of recurrent genetic alterations in BC development. We have focused our research on genes whose function is impaired or lost during BC development, with particular emphasis on chromosome regions 6q23-q25, 8p12-p21 and 17p13.3. Chromosome transfer studies using cell lines as target cells derived from breast carcinoma suggest the existence of TSG within these regions. Deletion fine mapping in combination with sequencing, sequence database searches and microarray technology have uncovered sequences of several candidate genes on 6q (i.e. MDC 11 and 12 genes), 8p (i.e. TRAIL receptor genes, EXTL3, LOXL2, BZF) and 17p13 (i.e. profilin 1) which are currently being tested. To validate breast cancer 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; mutation analysis. In addition, transfection assays and functional complementation tests have been applied.

Somatic genetic alterations in breast cancer: correlation of genetic data with clinicopathological parameters

S. Seitz, A. Nothnagel, K. Poppe, S. Werner in cooperation with P.M. Schlag (RRK, Berlin), J. Fischer (RRK, Berlin)

Specific genetic alterations could become new diagnostic markers for BC prognosis, providing defined individualized information about clinical outcome and response to therapy. In particular, LOH has been shown to occur commonly among various alleles at specific loci. A significant correlation was found between loss of 17p13 and parameters associated with more aggressive tumor behaviour, including large tumor size, grade, proliferative activity and estrogen receptor status. At present we are examining the contribution and prognostic relevance of genetic alterations on chromosome regions 8p, 8q, 6q and 17p at the genomic and transcriptional level.

Molecular pathology of solid tumors

K. Kölble, B. Barthel, H. Fischer, M. Simon

We have investigated the patterns of chromosomal and microsatellite instability in human tumors of the breast, gastrointestinal and urogenital tracts and have correlated these with the prevalence of germline and somatic mutations in a range of genes implicated in tumorigenesis (APC, CTNNB1, AXIN2). The development of a novel instrument and advanced methodologies for laser-assisted microdissection of human tissues and their integration with microscale real-time PCR and immunohistological expression analyses allows to detect quantitative expression patterns in morphologically selected small numbers of normal and neoplastic cells. Such multi-dimensional assays reflecting the various physiological and pathological differentiation states of tissues may help to refine the concepts and practice of molecular diagnostics.

Antibody engineering

B. Micheel, J. Schenk, G. Scharte

Antibody technology has been used to produce and modify antibodies against different antigens, including tumor-associated gene products. Several monoclonal antibodies have been produced against epitopes of the BRCA1 gene product. The antibodies have been tested by immunohistochemical techniques for their value as diagnostic reagents. Metabolic labelling experiments have been performed to screen for those antibodies reacting with the intracellular BRCA1 gene product that is biologically active. Binding partners of the BRCA1 gene product will be identified with the help of these antibodies. Monoclonal antibodies have also been produced against the products of newly identified tumor associated genes and these will be used to study the biological function of the gene products.

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Mouse Genetics

Carmen Birchmeier

We are using mice as a model organism for the functional analysis of genes important for development and disease. Tools for the molecular genetics are well established in mice, and homologous recombination and embryonal 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, now allows us to introduce subtle alterations like point mutations, or conditional mutations that are restricted to a particular cell lineage.

The role of Sox10 and the Neuregulin signaling system in development of neural crest cells and the peripheral nervous system

S. Britsch, M. Woldeyesus, A. Garratt, L. Li, M. Sieber

Neuregulin-1 is an EGF-like growth and differentiation factor that signals via tyrosine kinase receptors of the ErbB family. Two receptors, ErbB3 and ErbB4, bind Neuregulin-1 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. The third receptor, ErbB2, is present ubiquitously and acts as an essential co-receptor. The functional receptor complexes in vivo are thus 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 these mutations cause embryonic or postnatal lethality. We, therefore, also introduced more subtle mutations, for instance point mutations that eliminate either the alpha or beta isoform of Neuregulin, or conditional mutations in the ErbB2 receptor. Together, the analysis of these mutants revealed the important role of this signaling system in the development of neural crest cells and of the peripheral glial lineage.

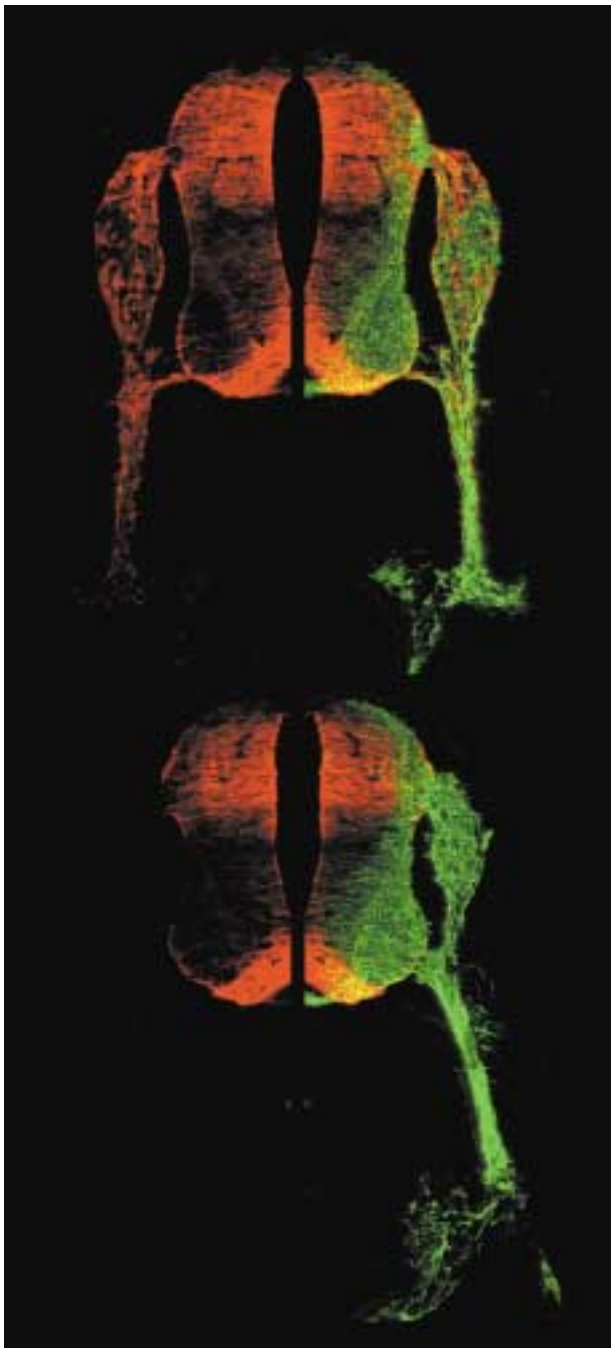
Animals with mutations in the Neuregulin/ErbB signaling system lack Schwann cells in the late developmental stages. The first changes observed in the development of the peripheral glial lineage appear very early, i.e. before differentiation of neural crest cells into early glial precursors. Since the sympathogenic neural crest sub-populations require Neuregulin/ErbB2 for migration, we have concluded that a deficit in migration of neural crest cells along the developing axons is the cause of this phenotype. Interestingly, work on the conditional mutants demonstrated that Neuregulin/ErbB2 has additional roles during subsequent stages of development of peripheral glial cells and that a single signaling system takes over multiple roles during the development of the lineage. Firstly, the early function in migration of neural crest cells, the precursors of the glial lineage; secondly, an essential role for growth and survival of early glial precursors, that allows an adjustment of cell numbers in the precursor pool. Upon terminal differentiation of the lineage, the glia becomes independent of Neuregulin/ErbB2 signals in terms of growth and survival. However, this is when the third role in myelination of Schwann cells becomes apparent.

Sox10 and ErbB3 have very similar expression patterns in neural crest cells and their derivatives. This prompted us to investigate a genetic interaction between the two genes. Following the analysis of Sox10 mutant mice, we found that appropriate ErbB3 expression in neural crest cells requires Sox10. Accordingly, Sox10 and ErbB3 mutant mice share phenotypes, for instance reduced numbers of neural crest cells that migrate along the outgrowing peripheral axons. However, analysis of the Sox10 mutants demonstrated that Sox10 has additional roles in development of the glial lineage, that are not mediated by ErbB3. Neuronal cells form in dorsal root ganglia in mice that carry a spontaneous or a targeted mutation of Sox10 but early Schwann cells or satellite cells are not generated. Sox10 is thus a key regulator of the differentiation of peripheral glial cells.

The role of the Neuregulin signaling system in heart development and function

C. Özcelik and A. Garratt

Mice with null-mutations in Neuregulin/ErbB2 display deficits in heart development, that cause embryonic lethality at mid-gestation. This genetic analysis revealed for the first time a role of Neuregulin/ErbB2 in cardiomyocytes, which was 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 develop 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 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. As in the development of the peripheral glial lineage, the signaling system is thus essential at distinct stages in the life of a cardiomyocyte. Conditional ErbB2 mutants provide a novel animal model of dilated cardiomyopathy, and will allow a rigorous assessment of the adverse effects of anti-ErbB2 antibodies on cardiac function.



Sox10 is essential for the differentiation of peripheral glial cells. Shown are cross sections through the spinal cord, dorsal root ganglia and spinal nerves of wild-type (upper image) and homozygous Sox10 mutant mice (lower image). Peripheral glial cells and neuronal cells are visualized with antibodies directed against B-FABP (red) and TuJ-1 (green), respectively. On the left side of each image only the red signal is shown; an overlay of the red and green signals is shown on the mirrored right side. Note that in homozygous Sox10 mutant animals, neuronal cells within the dorsal root ganglia form, but glial cells in the dorsal root ganglia and along the spinal nerves are not generated.

Genes that control migration of muscle precursor cells

H. Brohmann, M. Strehle, E. Vassioutina

Skeletal muscles in vertebrates, despite their functional and biochemical similarities, are generated by diverse developmental mechanisms. A major subclass of hypaxial muscle groups are derived from long-range migrating progenitor cells that delaminate from the dermomyotome. Our previous experiments have demonstrated that SF/HGF, its receptor c-Met and the adaptor, Gab1, are all essential for delamination of migrating myogenic precursors. A hypomorphic c-Met mutation and the mutation in Gab1 (the Gab1 gene encodes an adaptor important in c-Met signaling) reduce, but do not completely eliminate, c-Met signaling. Compound mutants in c-Met and Gab1 are able to “titrate” the signaling efficiency of the receptor further, without eliminating the signal completely. In such mutants, delamination of myogenic precursor cells occurs and, on occipital levels, precursors begin to migrate in the ventral direction. Instead of turning at a particular point along their route in an anterior direction, they stop migrating at this turning point, and appear to aggregate. Similarly, we have observed that myogenic precursor cells delaminate from somites at the forelimb level but they do not move distally and, instead, appear to aggregate in the proximal limb bud. We conclude from this that c-Met is not only essential for delamination, but also for migration of muscle precursor cells.
The homeobox gene Lbx1 has been identified as a mouse homologue of the Drosophila ladybird gene. During muscle development, expression of the Lbx1 gene is restricted to the migratory lineage. To analyze Lbx1 function during development, we have developed mice with a targeted mutation in the gene. In such Lbx1 mutants, migratory precursors form and delaminate from the dermomyotome, but migrate in an abnormal manner. Most strongly affected are those cells destined to move to the extremities, which fail to move laterally towards the limb buds and migrate ventrally instead. These misrouted cells accumulate in the mesoderm of the ventral body wall, and their migratory path is similar to that taken by cells that move towards the septum transversum, the analog of the diaphragm.

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Developmental Genetics

Andreas Schedl

Studying the molecular mechanisms underlying organ development is one of the key approaches to understanding the pathways leading to disease. We are particularly interested in two genes, the Wilms' Tumor suppressor WT1 and the HMG box-containing gene SOX9, both of which are involved in a number of genetic disorders in humans. Using transgenic and knock-out strategies in mice, we are analysing the molecular function of these transcription factors, developing model systems for human diseases and trying to understand the cellular and pathophysiological processes leading to the disease phenotype in mouse and man.

WT1 function in development and disease

WT1 is required for development of the adrenal gland and nephrogenesis.

Wilms' tumour is a paediatric kidney tumour occurring in 1 in every 10000 children. A proportion of patients show mutations in the Wilms' tumour suppressor WT1, which encodes a zinc finger protein with the ability to bind to both DNA and RNA. WT1 has also been implicated in embryonic development. Mice carrying homozygous mutations for WT1 fail to develop kidneys and gonads and die at day 13.5 in utero. We performed complementation experiments involving the mouse mutation by introducing human WT1 YAC constructs of 280kb or 470kb into the mouse genome. Both constructs rescued the knock-out mutation to different extents, which allowed us to look at WT1 function during later stages of development. Mice complemented with a 280kb construct survived until birth, but died within 48 hours due to defects in kidney development. Initial steps of nephrogenesis were completed, but mature epithelium never formed indicating a second role for WT1 during later stages of kidney development. Surprisingly, adrenal glands were also affected demonstrating the requirement of WT1 for this organ.

A crucial role for WT1 in the kidney podocyte: reduced expression levels cause glomerular disease

As described above, complementation experiments with the human 280kb construct were only partially successful, possibly because of the lack of a regulatory region on the construct used for our rescue experiments. Indeed, when using the 470kb human transgene, all developmental defects were complemented. Interestingly, rescued mice developed progressive glomerulosclerosis and died of renal failure a few months after birth. Using quantitative analysis we demonstrated that the observed phenotype is caused by too low levels of WT1 expression within the podocytes of the kidney. Moreover, we showed that nephrin and podocalyxin, two major components of the slit diaphragm of the podocyte, are dramatically downregulated in rescued animals, offering an attractive hypothesis for the molecular mechanism leading to renal failure. Our data have demonstrated for the first time an essential role for WT1 in the maintenance of podocyte function in adult life (Guo et al., in press).

Two splice variants of WT1 gene have distinct functions during sex determination and nephron formation

Alternative splicing of WT1 results in the production of various isoforms. Of particular interest is an alternative splice donor site at the end of exon 9, which introduces the three aminoacids KTS between zinc fingers 3 and 4. In vitro analyses indicated that + and -KTS isoforms fulfil distinct functions. Whereas –KTS isoforms are likely to act as transcriptional regulators, proteins harbouring the +KTS sequence have been proposed to act at the posttranscriptional level. To investigate these functions in vivo we have introduced subtle mutations into the endogenous Wt1 locus using ES cell technology. The point mutations were designed to interfere with splice donor site selection and, hence, result in the production of only the +KTS or –KTS allele from the targeted allele. Heterozygous mice carrying +KTS ablated ES-cells developed kidney abnormalities reminiscent of the Frasier/Denys-Drash syndrome (progressive mesangial sclerosis). When crossed to homozygosity +KTS mice survived embryonic development, but died perinatally possibly due to kidney failure. Podocytes completely failed to develop footprocesses demonstrating the importance of +KTS variants for the terminal differentiation of this cell type. Excitingly, these mice show male-to- female sex reversal and completely lack Sox9 expression in the developing gonad. In contrast, mice lacking –KTS isoforms (production of +KTS only) show streak gonads with a limited amount of Sox9. These data demonstrate a different role for the + and –KTS isoforms in vivo and place +KTS forms before SRY in the sex determination cascade.

SOX9 - a sex-determining gene

Mutations in SOX9 have been found in patients suffering from the semidominant campomelic dysplasia syndrome which is characterised by bowing of the long bones, XY sex reversal and perinatal death. The observed sex reversal suggests that SOX9 is required for male sex determination and, indeed, it is the first gene with a male-specific expression pattern after the expression of the sex-determining gene Sry. To address whether Sox9 is not only required, but also sufficient, to induce the sex determination pathway, we performed ectopic expression studies in which the mouse Sox9 locus was brought under control of Wt1 regulatory regions. Transgenic

mice generated with this construct expressed Sox9 in both male and female gonads. Interestingly, XX transgenic mice developed as phenotypical males and expressed male-specific markers such as MIS. Our results demonstrate that Sox9 is sufficient to induce the male sex determination pathway and suggests that Sry acts only as a molecular switch (Vidal et al., 2001).

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Lipids and Experimental Gene Therapy

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, FH), massive increase in the concentration of plasma lipoproteins results in hyperlipidemia and, as a consequence, 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. Focus of our studies is the elucidation of the various 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 study the consequences of the receptor gene defects *in vivo*.

Analysis of LDL receptor-related receptors

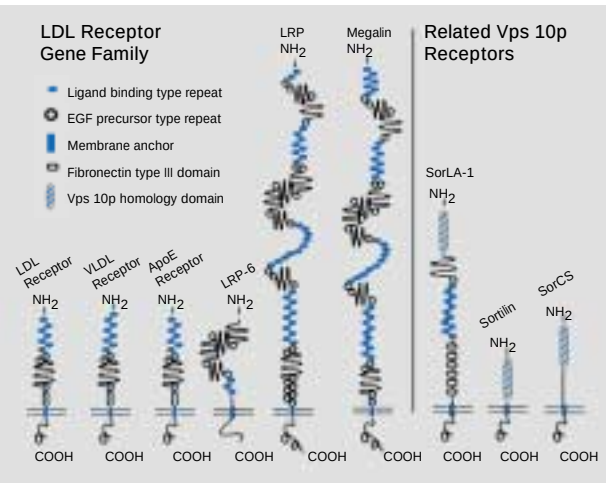
In recent studies, we have focused on the functional characterization of megalin, a member of the LDL receptor gene family predominantly expressed in the proximal tubules of the kidney. Experimental evidence suggested that this receptor is involved in the clearance of macromolecules from the glomerular filtrate. To test this hypothesis and to identify its endogenous ligands, we generated mice genetically deficient for the receptor and analyzed their tubular resorptive function. These studies identified megalin as a receptor for vitamin D binding protein (DBP), the plasma carrier for the steroid 25-(OH) vitamin D_3, and demonstrated that the receptor mediates the tubular retrieval of vitamin D_3-DBP-complexes filtered through the glomerulus. This receptor-mediated uptake is required to prevent the loss of vitamin D_3 metabolites by glomerular filtration. Furthermore, it delivers 25-(OH) vitamin D_3 to tubular epithelial cells for conversion into 1, 25-$(OH)_2$ vitamin D_3, the active form of the vitamin and a potent regulator of the systemic calcium and bone metabolism. Urinary excretion of 25-(OH) vitamin D_3 in megalin$^{-/-}$mice results in vitamin D deficiency and impaired bone calcification. Thus, megalin acts as an endocytic receptor for uptake of lipophilic vitamins and regulates a crucial step in the transport and renal conversion of vitamin D_3 metabolites. In parallel studies, we were able to identify patients that exhibit pathological symptoms highly reminiscent of murine megalin deficiency including urinary excretion of DBP, plasma vitamin D_3 deficiency and impaired calcification of bones. This finding suggests that megalin dysfunction may be the underlying cause of a human disease.

Ongoing research in our laboratory is directed towards the generation of new mouse models with obligate and conditional gene defects of LDL receptor-related receptors and the elucidation of their roles in the (patho)physiology of the cellular and systemic lipid metabolism.

Identification of endocytic receptors for lipophilic vitamins and steroid hormones

Mechanistically, the cellular uptake of 25-OH vitamin D_3-DBP-complexes by megalin resembles the endocytosis of lipoproteins by the LDL receptor and other lipoprotein receptors (figure 2). This observation suggests a common evolutionary origin of both lipid uptake pathways. In both cases, target cells express endocytic receptors that recognize a protein moiety, either a carrier protein, such as DBP, or an apoprotein embedded in a larger lipoprotein particle. After internalization of these complexes, the proteins are degraded in lysosomes while the lipid/vitamin moieties are transported into the cytosol where they are further metabolized. The identification of an endocytic pathway (megalin) for the steroid 25-OH vitamin D_3 may have important implications for our

Figure 1 Structural organization of some members of the LDL receptor gene family and related 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 domain (filled square), fibronectin type III repeat (open square) and Vps10p homology domain (filled oval).



understanding of the cellular metabolism of other steroid hormones. Because all steroid hormones are transported by specific plasma carrier proteins, similar endocytic uptake pathways may exist whereby bound steroids such as androgens and estrogens are specifically delivered to target cells. In particular, tissues that require large amounts of steroids, such as reproductive organs or steroid-responsive tumors, may use endocytosis to fulfill their needs for these essential regulators. In recent studies, we were able to identify additional endocytic receptors for DBP and for uteroglobin (Clara cell secretory protein), a proposed carrier for progesterone in the uterus and the lung, supporting a role of endocytic receptors in cellular steroid hormone uptake. In collaboration with industrial partners we are currently investigating the existence of additional pathways for the endocytic uptake of other steroid carrier proteins, and the role of the LDL receptor gene family in this process.

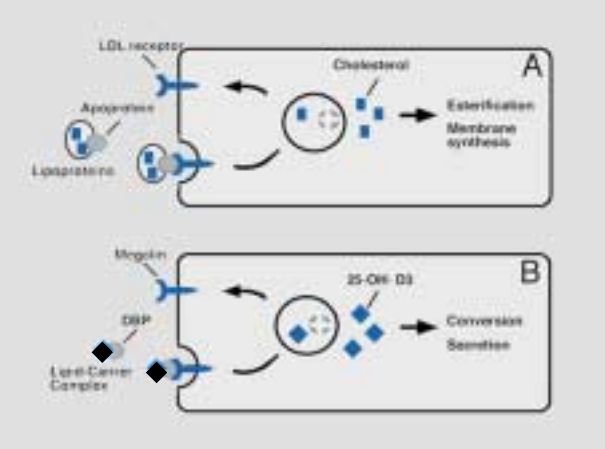


Figure 2 Endocytosis of lipoproteins and lipid-carrier-complexes by the LDL receptor gene family.
(A) Lipoproteins are taken up by the LDL receptor and other lipoprotein receptors via binding of the proteinaceous components of the lipoprotein particle, the apoproteins. The apoproteins are degraded in lysosomes, while cholesterol enters the cellular membrane pool or is stored as cholesterol esters. (B) Lipid-carrier-complexes are internalized via megalin through recognition of the carrier protein DBP. The carrier is degraded while the bound steroid 25-OH vitamin D_3 is converted into 1,25-$(OH)_2$ vitamin D_3 and re-secreted.

Functional characterization of cellular sorting receptors

Previously identified members of the LDL receptor gene family solely 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 and in the mannose 6-phosphate receptor (figure 1). SorLA-1 in turn is highly homologous to a novel class of mammalian Vps10p-related receptors designated sortilins or sorCS (figure 1). The physiological role of these sorting receptors and their relevance for the activities of the LDL receptor gene family is unclear at present. However, expression of the receptors in kidney, adipose tissue and heart suggests important functions in the cardiovascular systems. We have generated knockout mouse models lacking functional expression of the various members of this gene family and are currently working on the phenotypic analysis of these animal models.

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

Two subgroups of our team (genome analysis, P.B., metabolic pathway analysis, S. Schuster, and lipid network, J.R.) continued in a joint endeavour to evaluate the importance of SNPs and splicing variants in the human genome. This was combined with an application study on 1250 normal probands (recruited by INFOGEN-VALIGEN Medical Genetics GmbH [H. Schuster], and Franz-Volhard-Clinic, FVK [F. Luft]), in which 30 SNP variants at 6 relevant gene loci (for names and abbreviations see figure legend) 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 for 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 (myocardial infarction, stroke, a.o.). 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 FVK under standardized conditions and SNP genotyping was done by VALIGEN´s genomic center in Paris. 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 (S. Schuster et al. 2000, H. Knoblauch et al., 2000). The data obtained were subjected to an exhaustive mathematical-statistical analysis using advanced computer techniques.

The main result is that 25 of 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 (incidentally: Peer Bork and two further members of our group are the only German theorists co-authoring the milestone NATURE paper of Lander et al. that published the full reference sequence of the human genome on February 15th , see publication list). About half of the SNPs were in the coding region (non-synonymous and synonymous) the others in promoter and intron segments.

We found that within 1 kbp distance (= 1000 nucleotide letters) all SNP pairs showed complete LD, since only 2 or 3 of the 4 possible 2-SNP haplotypes were found, pointing to the absence of recombination effects in the population at such small inter-SNP distance. At larger distances (our gene loci span between 30 and 140 kbp on the genome) the LD levels off and reaches full equilibrium due to recombination at about 50 kbp.

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 mapping of functional gene alleles only at small distances from the marker SNP. However, we could establish the SNP-haplotype structure of all loci by way of analysis of the family pedigrees of the 1250 subjects, since they were recruited as nuclear families in a systematic field working project of INFOGEN. Haplotyping was achieved with a newly developed computer program (Rohde & Fürst, 2001) that permits the establishment, with high confidence, 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 4 of the studied gene loci only a few (4-5) haplotypes accounted for the genotype of about 80-90% of the population sample (see fig.). 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 families and within families permit to control for stratification (sample inhomogeneity) effects.

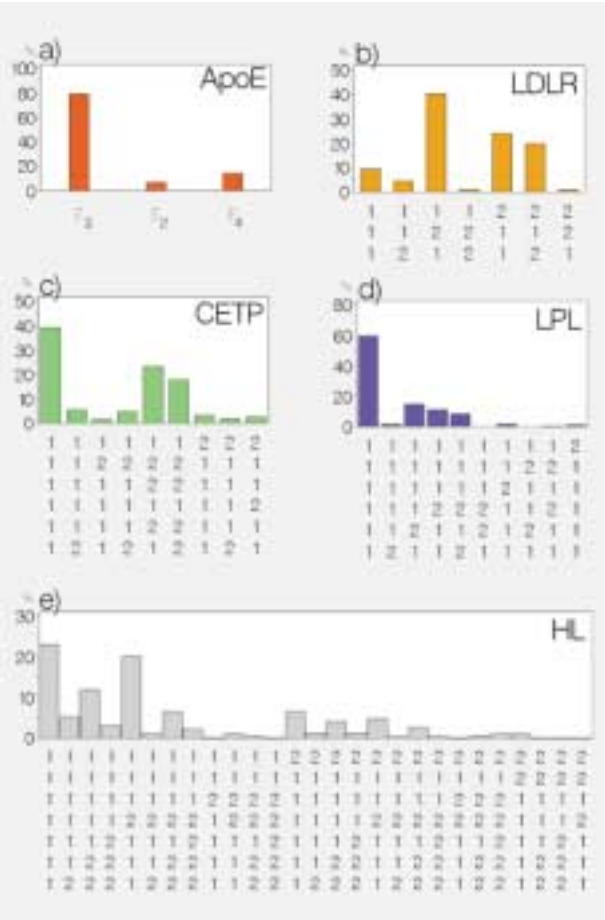
We found that at 5 of the loci there are chromosomal haplotypes in the population which change the mean lipid levels of their carriers in a significant way. Most impressive is the variation effect of apolipoprotein E (ApoE), which confirms results published for Sweden, America and other countries. ApoE, whose variants play a major role as risk or protection factors in lipid disorders as well as in the causation of Alzhei-

mer disease, explains about 5-10% of the global variance of the lipid traits in normal people. The contribution of SNPs at the other loci, notably lipoprotein lipase, hepatic lipase, cholesteryl ester transfer protein and the famous LDL receptor, was demonstrated for the first time. Their effect is less pronounced, but still significant. The SNP haplotype variation of all 5 variant loci studied by us explains between 10 and 20% of the whole variance and amounts therefore to one third to one half of the genetic inheritance that was established in the twin studies.

The results (to be 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. Before publication we will have to enlarge the sample and include some relevant loci (apoproteinA, apoproteinB) for which hitherto no SNP variants were obtained simultaneously with lipid values.

Genetic diversity of lipid genes in the German population

SNP-haplotype frequencies from 616 chromosomes of unrelated persons, coded 1 (= more frequent SNP-variant) and 2 (= less frequent SNP-variant) from 5' to 3' on the genome: a) apolipoprotein E (ApoE), b) low density lipoprotein cholesterol receptor (LDLR), c) cholesteryl ester transfer protein (CETP), d) lipoprotein lipase (LPL), e) hepatic lipase (HL).



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Protein Misfolding: From Basic Biopolymer Physics to Conformational Diseases

Gregor Damaschun

Amyloid fibers are the characteristic common to a number of so-called protein conformational disorders. Systemic or organ-specific amyloidoses, including Alzheimer's disease, and the transmissible spongiform encephalopathies belong to this class of disorders. More than twenty of these human and animal diseases are known where a specific protein is misfolded to form amyloid fibers in each case. In spite of the very different proteins involved, the amyloid fibers have very similar conformations. Slow kinetics of progression is typical of all amyloidoses and the process of fiber formation is only poorly understood up to now.

Protein conformational disorders can be promoted by point mutations but appear also spontaneously or are caused by infectious protein conformations (prions).

In recent years, it has been observed that about thirty proteins unrelated to so far known diseases can form amyloid fibers.

This observation has led to the suggestion that the ability to form amyloid fibers is a common phenomenon and a generic property of polypeptide chains. The ability to form amyloid fibrils from a wide range of proteins allows a large number of model systems to be used to study the process of fibril formation in more detail.

We have recognized that the adage "one sequence, one conformation" is not strictly true. Depending on solvent conditions at folding, probably any protein chain can adopt a variety of conformations in which there is a degree of periodic order, i.e., extensive-regions of secondary structure. A recent systematic study of the conformations adopted by the glycolytic enzyme, phosphoglycerate kinase, shows that in different media the chain can adopt five distinct states *in vitro*. However, such alternative states do not have the precisely and tightly packed side-chains, which are the hallmark of the native state of orthodox globular proteins.
One of these alternatives is the amyloid state, the result of protein conformational disorders *in vivo*.

Aβ-Amyloids

(in cooperation with E. Krause, FMP)

A critical event in Alzheimer's disease is the transition of Aβ peptides from their soluble forms into disease-associated β-sheet-rich conformers. Structural analysis of a complete D-amino acid replacement set of Aβ(1-42) has enabled us to localize in the full-length 42-mer peptide the region responsible for the conformational switch into a β-sheet structure. Although NMR spectroscopy of trifluoroethanol-stabilized monomeric Aβ(1-42) revealed two separated helical domains, only the destabilization of helix I, comprising residues 11-24, caused a transition to a β-sheet structure. This conformational α-to-β switch was directly accompanied by an aggregation process leading to the formation of amyloid fibrils.

Kinetics of amyloid formation

(in cooperation with H. Fabian, MDC)

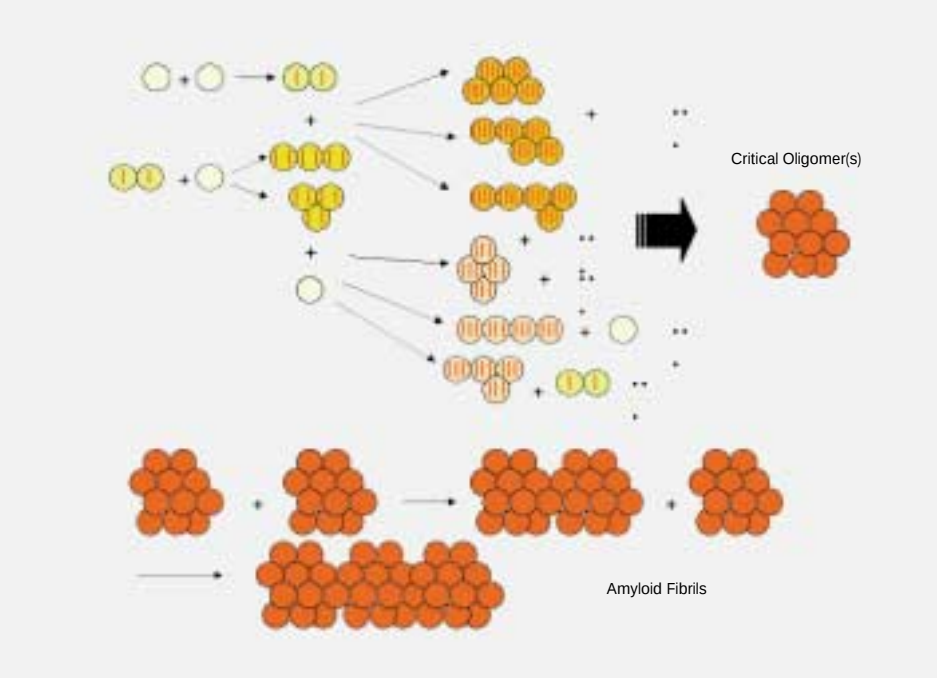
We have studied the transformation of different model proteins into the amyloid conformation by time-resolved simultaneous measurements by means of dynamic and static light scattering, X-ray scattering, circular dichroism and Fourier-transform infrared spectroscopy. This combination of methods allows us to determine simultaneously changes in particle dimensions and mass as well as changes in the secondary structure.

The main results are the following. Monomers having the typical amyloid-like cross-β structure do not exist as was postulated by Prusiner for the prion. The conformational change from α-helices into β-sheets is triggered by the onset of monomer aggregation. The amount of β-structure grows until the formation of an ensemble of critical oligomers is finished. These critical oligomers assemble into wormlike amyloid fibers in a second step. The fractal dimensions of the two growth mechanisms are different.

Protein misfolding and structure transformation

Protein molecules adopt their native conformation in solution and within the cell only under specific environmental conditions. Alcohols are suitable for modulating the interactions between the polypeptide chain and the solvent as well as the interaction between different segments of the polypeptide chain. Recent investigations have dealt with the influence of cosolvents on the folding kinetics of proteins, the tuning of solvent conditions for studies of the amyloid formation of peptides and proteins, the dissection and reassembly of amyloid fibrils, and the α-β-transition of proteins, which plays an essential role in various conformational diseases.

The conformational transitions of bovine β-lactoglobulin A and phosphoglycerate kinase from yeast induced by hexafluoroisopropanol (HFIP) and trifluoroethanol (TFE) have been studied by dynamic light scattering and circular dichroism spectroscopy in order to elucidate the potential of fluoroalcohols to bring about structural changes in proteins. Moreover, pure fluoroalcohol-water mixed solvents were investigated to prove the relationship between hydrophobic cluster formation



Coagulation model for the formation of amyloid fibers from misfolded protein molecules. The blue bars and circular areas represent the increasing amount of newly formed cross-β structure of the polypeptide chains. Cross-β structure is typical of amyloid fibers. The conformational transformation is caused by aggregation.

and the effects on proteins. The results demonstrate that cluster formation is mostly an accompanying phenomenon because important structural changes in the proteins occur well below the critical concentration of fluoroalcohol at which the formation of clusters occurs. According to our light scattering experiments, the remarkable potential of HFIP is a consequence of extensive preferential binding. Surprisingly, preferential binding seems to play a vanishing minor role in the case of TFE. However, the comparable Stokes radii of both proteins in the highly helical state induced by either HFIP or TFE point to a similar degree of solvation in both mixed solvents. This shows that both direct binding and an indirect mechanism must be taken into consideration to explain the effects of alcohols on proteins.

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

Udo Heinemann

Macromolecular crystallography is a uniquely powerful tool to study the three-dimensional structures of proteins, nucleic acids and their complexes. It permits to determine the precise arrangement of all atoms and the shape and property of all surfaces in small or medium-sized 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 given protein molecule. We combine X-ray diffraction studies with biochemical and biophysical experiments of proteins involved in a variety of biological processes. 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 scientific infra-structure at our disposal is currently being extended with the completion of a state-of-the-art protein crystallography station at the electron storage ring BESSY II in Berlin-Adlershof.

Below, selected examples from the different areas of our research are presented. They include work by Yves Muller, who has recently accepted an offer to join the faculty of the University of Sussex, UK, and his colleagues.

Nucleic acid-interacting proteins

R. Bienert, H. Delbrück, D. Khare, Y. Roske, T. Schwartz, E. Werner

Recent work in the group of A. Rich (MIT, Cambridge, USA) had shown that the unusual left-handed Z-form of DNA can be induced and stabilized by binding of the Zα domain of the RNA-editing enzyme ADAR1. To examine whether ADAR1 is part of a larger family of Z-DNA-binding proteins, the weakly sequence-related N-terminal domain of the tumor-associated protein DLM-1 from mouse was bound to a self-complementary six-base-pair oligonucleotide and crystallized. The crystal structure of this $Z\alpha_{DLM}$-DNA complex clearly showed the DNA to adopt the Z-conformation. $Z\alpha_{DLM}$, as well as $Z\alpha_{ADAR}$ bind the DNA conformation- rather than sequence-specifically by contacting mainly the uniquely shaped sugar-phosphate backbone. The single base contact made is with the C8 atom of a guanine which is exposed for intermolecular contact only in the Z-form. The crystal structure of the $Z\alpha_{DLM}$-Z-DNA complex thus proves the existence of a family of Z-DNA-binding proteins which includes further molecules such as the vaccinia virus protein E3L. In which way these proteins use the B↔Z transition of DNA to modulate biological processes remains to be established.

Electron transport in cytochrome P450 systems

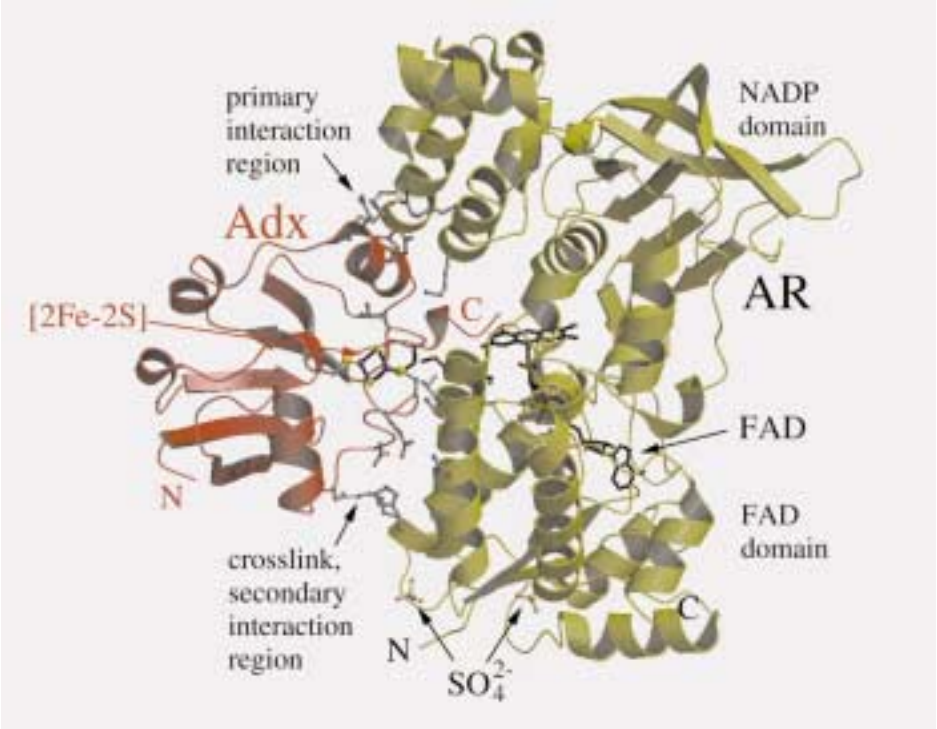
J. J. Müller

To continue our studies of electron transport to cytochromes P450, carried out in collaboration with K. Ruckpaul (MDC) and R. Bernhardt (Universität des Saarlandes), a complex of the [2Fe-2S] ferredoxin adrenodoxin cross-linked to its cognate adrenodoxin reductase was crystallized. The structure analysis of this complex was a challenge, since the crystals displayed an unusually long primitive axis of >600 Å. The structure (see figure) confirmed biochemical data regarding residues of adrenodoxin involved in reductase binding and identified two additional interaction regions of the ferredoxin. A probable route for the electron transfer from the FAD of adrenodoxin reductase to the iron cluster of adrenodoxin could be delineated based on the crystal structure.

Structural basis of protein stability

H. Delbrück, U. Mueller

The small, bacterial cold shock proteins (Csp's) are extremely well suited for studies of the structural basis of protein stability, since they fold in a reversible two-state process and their analysis is not complicated by disulfide bonds, bound cofactors or multimerization. In collaboration with F.X. Schmid (Universität Bayreuth) we have studied two Csp's from different *Bacillus* strains that differ in only a small number of amino-acid side chains but show a large difference in thermal stability. We could show that the backbone structure of these proteins is virtually unchanged and the stability difference is mainly caused by electro-static interactions between side chains. Studying a set of Csp mutants it could be shown by biophysical experiments that only two surface residues are responsible for the entire stability difference and by crystal structure analysis that these side chains do not engage in defined pairwise salt bridges but rather help establish the global surace charge balance of the proteins. This work permits the important conclusion that proteins may be easier stabilized by the removal of electrostatic repulsion between side chains than the engineering of salt bridges.



Crystal structure of the electron-transfer complex formed between adrenodoxin (Adx) and adrenodoxin reductase (AR). The redox-active groups of the two proteins and the amino-acid side chains involved in complex formation are shown in a schematic drawing of the molecules. From Müller et al. (2001).

LG domains and their functions

I. Grishkovskaya, G. Sklenar, Y. A. Muller

Laminin G-like domains (LG domains), also called "LNS" domains, are commonly found building blocks in extracellular proteins, where they bind a diverse range of ligands and are involved in a host of biological functions. The comparison of the crystal structure of sex hormone-binding globulin (SHBG) solved at MDC with the LG/LNS domains from neurexin and laminin α2 chain revealed a close structural relationship between LG domains and legume lectins. Of these proteins, SHBG appears to be a rather complex molecule. The crystal structures of various steroid complexes reveal that androgens and estrogens are bound in different orientations in the steroid-binding pocket. Steroid binding is modulated by zinc. The crystal structures of SHBG in presence and absence of zinc show how the binding of zinc to a site near the steroid site induces conformational disorder in a loop segment that covers the steroid.

The cell surface receptors tissue factor and sky

K. Faelber, C. Heiring, Y. A. Muller

The outstanding importance of the antigen-antibody recognition process for the survival and defence strategy of higher organisms is in sharp contrast to the limited number of high-resolution structures available on antibody-antigen pairs with antigenic proteins. We solved the crystal structure of the complex between tissue factor (TF) and the humanized Fab fragment D3h44 at high resolution as well as the structure of uncomplexed D3h44. This gave us a unique opportunity to study the recognition process in detail. We observed that upon complex formation conformational changes are very small and almost exclusively limited to the reorientation of the side chains. Presently our focus has shifted to structural studies on the sky receptor. The sky receptor belongs to the Tyro-3 receptor tyrosine kinase family and is activated by the ligand Gas6.

Structural genomics

T. Niedenzu

A recently established international initiative to determine the three-dimensional structures of a set of protein molecules representing all known sequence families has been given the name structural genomics. Our group has assumed a leading role in the Berlin-based structural genomics project, the Protein Structure Factory, which is at the forefront of research in this area. The Factory is setting up an infrastructure for high-throughput protein structure analysis by X-ray crystallography and NMR spectroscopy. Important parts of the Factory are centralized expression cloning and protein purification facilities, a large robotic station for protein crystallization and protein crystallography beamlines at the electron storage ring BESSY II in Berlin-Adlershof.

Selected Publications

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Role of Protein Dynamics in Enzyme Function

Christiane Jung

The dynamic behavior of protein structures and its relationship to protein folding and function are the main focus of our research group. The thiolate heme proteins, cytochrome P450 and NO synthase, are the main subjects studied. While P450s are involved in several metabolic processes in animal and human organs, e.g. the biotransformation of drugs and the biosynthesis of steroid hormones, the NO synthases play a critical role in the production of the important signal molecule NO. Both enzymes have a very similar heme-iron coordination sphere and analogous intermediate steps in the reaction cycle, although their function, protein structure and interaction with redox partners are very different. Uncovering the fundamental structural requirements for this different behavior will contribute to a better understanding of the reaction mechanisms and to the development of new strategies for the design of enzyme inhibitors of great medical significance.

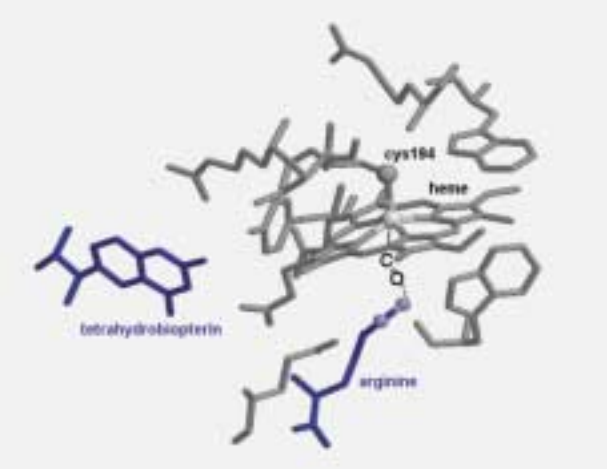
In the last two years we have continued our studies of the structural analysis of cytochrome P450s from various sources and of inducible NO synthase using various techniques.

Structural changes implicated in the reduction of Cytochrome P450

There are two important steps in the reaction cycle which are involved in the reduction process - these are redox-partner complex formation and electron transfer to the heme iron. Using Fourier-transform infrared spectroscopy (FTIR) we have resolved changes in the protein structure occuring in both steps. In the first step, the iron-sulfur protein putidaredoxin (Pdx) is the natural redox partner for cytochrome P450-cam. If Pdx is titrated by P450cam and analyzed by FTIR and principal-component analysis, an infrared difference spectrum is obtained which clearly indicates structural changes in the protein and amino-acid side-chains. We found that there was a salt-link formation between an arginine and an aspartate. In addition, there was deprotonation of a glutamate and changes in turn-regions of the secondary structure. This example shows that we have successfully established a new approach to studying protein-protein interactions by FTIR. For the second step, we previously showed that photoreduction is an appropriate tool to uncover structural differences between the oxidized and reduced form of cytochrome P450. Using ruthenium complexes covalently bound to amino acid side-chains on the protein surface, a long-range intramolecular electron transfer over 27 Å to the heme iron was detected by FTIR spectroscopy. The structural changes connected with this process are similar to those observed for photoreduction with unbound ruthenium complexes.

Reaction intermediates of cytochrome P450

The reaction cycle of thiolate heme proteins procedes via an unstable intermediate, called compound I or [Fe-O] species. For many years it was assumed that this intermediate was similar to the corresponding intermediates of peroxidases. In collaboration with the group of A.X. Trautwein, Medical University of Lübeck, we have shown for the first time that chloroperoxidase and cytochrome P450cam have a different intermediate. This intermediate has been produced and stabilized by freeze-quench experiments and characterized by Mössbauer and EPR spectroscopy. It turns out that the heme iron is in the Fe(IV) state. In addition, a tyrosine radical has been found for P450cam in contrast to the porphyrin π-cation radical in chloroperoxidase. These results have made an important impact on our understanding of the complex reaction mechanism for thiolate heme proteins.



Active-site structure of iNO synthase indicating the hydrogen bond between the guanidinium nitrogen atom of the arginine substrate and the heme iron CO ligand (PDB entry code 1nod, the CO ligand is sketched).

Active-site structure of NO synthase

In cooperation with D.K. Ghosh, Duke University Medical Center, Durham, USA, and G. Guillemette, Dept. of Chemistry, University of Waterloo, Canada, we have analyzed the effect of arginine and tetrahydrobiopterin on the active-site structure of inducible NO synthase (oxygenase domain) from mouse and human full-length iNOS using FTIR. The stretch mode of the heme iron CO ligand was used as a spectroscopic

probe. We found that arginine forms a hydrogen bond with the CO ligand (Figure). High-pressure FTIR using a membrane-driven sapphire-anvil cell allowed us to study the pressure-induced shift of the CO stretch mode and the compressibility of the active site was determined. It turns out that the active-site structure in arginine-bound iNOSox is less compressible than in camphor-bound P450cam.

Selected Publications

Jung, C. (2000). Insight into protein structure and protein-ligand recognition by Fourier transform infrared spectroscopy. J. Mol. Recogn. 13, 325-351.

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Simgen, B., Contzen, J., Schwarzer, R., Bernhardt, R., and Jung, C. (2000). Substrate binding to 15β-hydroxylase (CYP106A2) probed by FT infrared spectroscopic studies of the iron ligand CO stretch vibration. Biochem. Biophys. Res. Commun. 269, 737-742.

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Computer Simulation of Nucleic Acid Structure and Interactions

Heinz Sklenar

Understanding nucleic acid structures is important due to the variety of biological functions fulfilled by DNA and different classes of RNA molecules. To contribute to the functional annotation of regulatory regions in genomic sequences we have focussed on the development of both structural analysis tools and new simulation techniques. 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, with the advantage that the full sequence space can be readily explored.

Structural analysis tools

The accumulation of structural data on nucleic acid fragments and nucleic acid complexes with proteins and other molecules has emphasized the need for clear and standardized analysis tools. In the case of helical structures, helical parameters make important geometrical features easily readable, provided they faithfully quantify sequence-dependent helical deformations which are generally present. The CURVES approach satisfies this goal by providing a full set of helical parameters and by defining an unique, generally curvilinear, helical axis.

The revised version (CURVES 2000) takes into account the decisions from the 1999 Tsukuba meeting on DNA structural analysis and the new IUPAC-IUBMB approved base coordinate system (Olson et al. (2001) J. Mol. Biol. 313, 229-237). The new program is both simpler from a mathematical point of view and easier to use, and should avoid confusion between local and global helical parameters. It has also been extended to include a detailed analysis of groove geometry, accompanied by new graphical representations.

Functional annotation of regulatory regions in genomic sequences

Subtle sequence effects on the helical geometry of DNA 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 are participating in an international initiative started during the expert meeting in Ascona 2001, with the goal to improve the underlying data by using large-scale Molecular Dynamics simulations on the current state-of-the-art level.

Dynamics of DNA and RNA structures

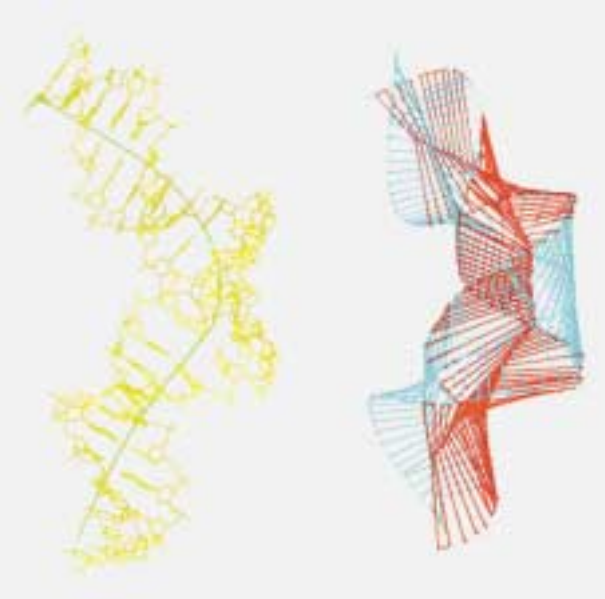
Besides the detailed atomic resolution structure an understanding of the mobility and conformational deformability of DNA and RNA molecules is important for interpreting their function. Currently, only some aspects of the dynamic behavior of nucleic acids can be measured experimentally. In a detailed study on the conformational deformability of Watson-Crick paired, mismatch and bulge containing RNA fragments, the harmonic-mode analysis method has been used. To overcome the limitations of this method we have now focussed on the development of a practicable simulation technique using the Monte Carlo Metropolis algorithm. In contrast to the more rigorous Molecular Dynamics approach, the new technique is computationally very fast. First encouraging results show experimentally known conformational conversions of the sugar-phosphate backbone as frequently observed events, and B-A-B transitions of DNA can easily be induced by changing solvent conditions. Thus, even folding simulations of small RNA fragments could become feasible by means of this method.

DNA-ligand interactions

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 conditions. The relative stability of six alternative structures, derived from our former modeling study of methylene blue binding to a DNA decamer with alternating GC base sequence, has been analyzed as a function of salt concentration. The results of finite difference numerical solutions of the nonlinear Poisson-Boltzmann equation show that the stabilizing effect of salt is larger for free DNA than for the modeled complexes. Accordingly, the estimated binding energies decrease with increasing ionic strength. A slightly higher stabilization of the groove binding complexes results in comparable binding energies for symmetric intercalation and minor groove binding at high salt concentration. Both results are in agreement with experimental data.

Conservation and diversification of C2H2 zinc finger proteins in eukaryotic genomes

C2H2 zinc fingers, short repetitive sequence modules in zinc finger proteins, are the most frequent nucleic acid binding motifs in eukaryotic genomes. Their high sequence variability, combined with different arrangements of a variable number of fingers, results in rather diverse functions of the zinc finger proteins, ranging from sequence-specific binding to DNA or DNA/RNA hybrids, binding to RNA or non B-form DNA, to their involvement in protein-protein interactions. Using a substantially improved Pfam search pattern, developed in joint work with the Sanger Centre (UK), the complete sets of zinc finger proteins have been identified in the genomes of *Arabidopsis*, yeast, C. *elegans*, *Drosophila* and man. Comparative sequence analysis of these five sets has revealed a new level of zinc finger protein complexity due to their diversification in evolution. 90% of the zinc finger proteins encoded by the *Arabidopsis* and yeast genomes are species specific and not found in the three other genomes. 75% of C. *elegans* and *Drosophila* zinc finger proteins are species specific as well and not conserved in man. This diversification is mainly caused by independent expansion of the zinc finger proteins in each genome of the major eukaryotic lineages. Most of the zinc finger proteins conserved between invertebrates and man belong to subfamilies of DNA binding transcription factors. However, several of these are also expanded independently in the different species, with an increasing number of family members in the order *C. elegans* < *Drosophila* < man.



Graphical representation of the CURVES analysis for a DNA fragment bound to the TATA box binding protein, showing the global curvilinear helical axis (green) and the calculated major (red) and minor (blue) groove geometry.

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Rohs, R., and Sklenar, H. (2001). Methylene Blue binding to DNA with alternating GC Base sequence: Continuum treatment of salt effects. Ind. J. Biochem. Biophys. 38, 1-6.

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Conformation, Stability and Interaction of Biological Macromolecules

Heinz Welfle

The main areas of our research involve studies of the physicochemical properties of nucleic acid-binding proteins and their complexes with RNA or DNA, and of antibodies and antibody-peptide complexes. The main tools used are circular dichroism, fluorescence, infrared and Raman spectroscopy, and calorimetric methods.

Antibody-peptide interactions

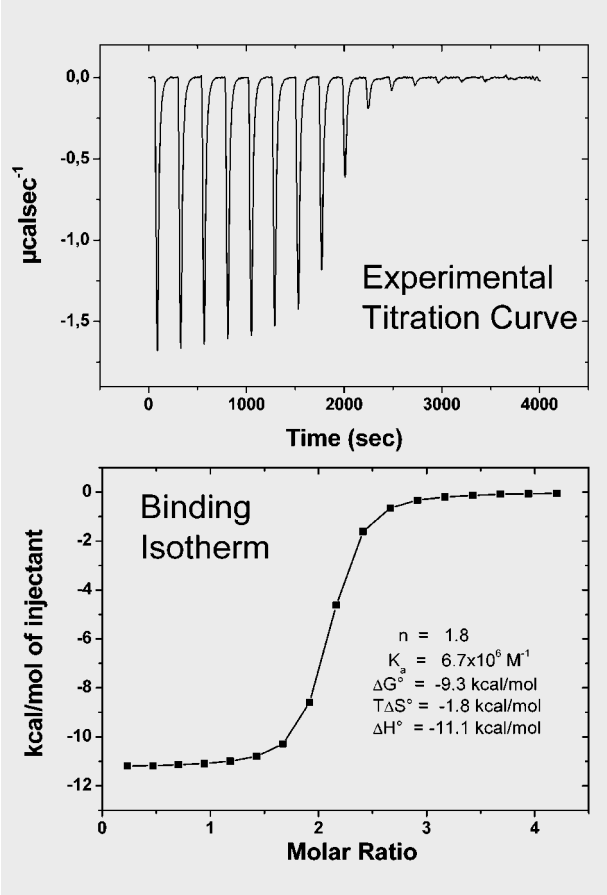
Essentially the same features determine the recognition of proteins and peptides by antibodies. However, the usually unfolded and flexible structure of peptide ligands in solution complicates the first steps of recognition; large changes in the conformational entropy contribute to the energetics of the binding reaction. Isothermal titration calorimetry provides an experimental tool for the investigation of the free energy of binding, $\Delta G°$, separating it into enthalpic and entropic components. In collaboration with Wolfgang Höhne and Jens Schneider-Mergener, Humboldt-Universität Berlin, we have studied in detail the binding of peptides by high affinity antibodies CB/RS/1 (anti-interleukin 10 monoclonal antibody), CB4-1 (anti-p24 (HIV-1) monoclonal antibody), and tAB2 (anti-TGFα monoclonal antibody). Although monospecific interaction of an endogeneous epitope is expected for the physiological function of antibodies, antibodies can bind to homologous and non-homologous peptides; high affinity binders were identified by screening of large combinatorial peptide libraries. For example, the murine anti-p24 (HIV-1) antibody CB4-1, binds to a linear peptide epitope of the capsid protein and also to several unrelated peptides. X-ray crystal structure analyses show that these peptides assume different backbone conformations and utilize different interactions in CB4-1 Fab fragment complexes with epitope-homologous and non-homologous peptides (T. Keitel, A. Kramer, H. Wessner, C. Scholz, J. Schneider-Mergener, Cell 91, 1997, 811-820). Binding constants, binding stoichiometry and binding enthalpies were obtained experimentally to allow the calculation of binding free energies and binding entropies. For the antibody-peptide interactions studied so far, enthalpy and entropy contributions to the free energy differ significantly, but in each system the complex formation is enthalpically driven.

Translational initiation factor IF2 from *Bacillus stearothermophilus*

Initiation factor IF2 is involved in the initiation step of eubacterial translation, and its main known function is the correct positioning of initiator fMet-tRNA^{fMet} in the ribosomal P site. In collaboration with C.O. Gualerzi, University of Camerino, Italy, and U. Heinemann, MDC, we have provided clear evidence for the localization of the entire fMet-tRNA^{fMet} binding site in a small C-terminal subdomain, IF2 C-2, from the *Bacillus stearothermophilus* factor. Our earlier protein unfolding studies had suggested that IF2 C, the 24.5-kDa fMet-tRNA binding domain of IF2, may consist of two subdomains. Replacement of the four Phe residues of IF2 C yielded four variant proteins having intrinsic fluorescence markers in different positions of the molecule. Comparison of the circular dichroism and Trp fluorescence changes induced by increasing concentrations of guanidine hydrochloride demonstrated that IF2 C indeed consists of two subdomains: the more stable N-terminal (IF2 C-1) subdomain and the less stable C-terminal (IF2 C-2) subdomain. Isolated subdomain C-2, which consists of just 110 amino acids, was found to bind fMet-tRNA^{fMet} with the same specificity and affinity as native IF2 or IF2 C-domain. Characterization of IF2 C-2 by circular dichroism, by urea-, guanidine hydrochloride-, and temperature-induced unfolding, and by differential scanning calorimetry indicates the properties of a globular molecule containing predominantly β structures (33%) and turns (19%). Complex formation between fMet-tRNA^{fMet} and IF2 C or IF2 C-2 is accompanied by barely detectable spectral changes as demonstrated by a comparison of the Raman spectra of the complexes with the calculated sum of the spectra of the individual components. These results and the temperature-dependence of the K_d of the protein-RNA complexes, determined by analytical ultracentrifugation studies, indicate that complex formation is not accompanied by obvious conformational changes in the components and, possibly, depends on a rather small binding site comprising only a few interacting residues of the two components.

Plasmid pSM19035-encoded proteins

Because of the central role of the pSM19035-encoded ω-protein in regulating the maintenance of low-copy-number plasmids, we initiated a detailed analysis of this protein. The longterm goal of this project is to elucidate, in collaboration with W. Saenger, FU Berlin, and J. Alonso, C.S.I.C. Madrid, the cellular systems that ensure stable inheritance of low-copy-number plasmids within bacterial cells by an accurate control of DNA replication and an ordered partition at cell division. pSM19035-encoded products that avoid the appearance of plasmid-free cells, map within the SegA and SegB regions. The SegB region, that makes plasmids ~1000 times more stable than expected for random segregation, includes the δ, ω, ε and ζ genes. At the transcriptional level, ω protein, the product of the ω gene, coordinates the expression of pro-



Titration of monoclonal anti-p24 (HIV-1) antibody CB4-1 with peptide ATPQDLNTn-LeuL. Titration was performed at 25°C in 50 mM sodium phosphate, pH 7.2, injecting 100 µl of 0.53 mM peptide solution in 5 µl steps into 0.016 mM antibody solution. The binding isotherm is calculated from the experimental data and provides the number of binding sites n, the binding constant K_a, the free energy $\Delta G°$, the entropy $T\Delta S°$ and the enthalpy $\Delta H°$ of binding.

teins required to control the copy number and maintainance of plasmids. Using circular dichroism, fluorescence spectroscopy, analytical ultracentrifugation and an electrophoretic mobility shift assay, the wild type ω protein and a variant with a C-terminal hexa-histidine tag (ω-H_6) were characterized. The ω-protein is mainly α-helical (42%), occurs as a dimer in solution, unfolds thermally with half transition temperatures, T_m, between ~43 and ~78°C depending on the ionic strength of the buffer, and binds P_{cop}S DNA with high affinity. Further studies will focus on DNA binding properties of the ω protein.

Selected Publications

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At the beginning of 2002 a new research unit was established at MDC to collaborate closely with a sociological research group from the Research Center Juelich (Forschungszentrum Jülich, FZJ) on the project "Discourse on the Ethical Questions of Biomedicine". The project is funded by the Federal Ministry for Education and Research.

Brief description of the project

- A public initiative designed to serve as a model of a biomedical research centre in close collaboration with a sociological unit of the FZJ on matters involving public information, dialog and consensus processes concerning ethically relevant biomedical questions of today
- Testing and exploitation of a variety of structured discourse options to assist in the formation of public opinion/consensus building over a wide range of issues and at various levels
- The testing of new communication media for discussions (Internet discussion)
- A one-year scientific and interdisciplinary discussion process accompanied and moderated by experts and "advisers" from specific social groups on the subject of "Ethical questions regarding research on human stem cells and their medical exploitation"
- Implementation of a conference of laymen on the same subject
- Scientific evaluation and further development of the applied procedures to help resolve value conflicts in society

Target discussion groups

- The general public throughout the Federal Republic
- Decision-makers from politics and business
- Scientists and other experts

Principles

The guiding principle of the project is a permanent, informed discourse between researchers and the public which is structured towards creating consensus processes. In this connection, the group aims to develop an interface for German biomedicine that will serve as an example in the future. This complex and long-term project will take place in close association with the MDC and its staff and take direct advantage of their expertise. However, it will not be thematically limited to research matters of interest to the MDC, but will extend beyond this and deal with the issues in a complex and interlinked manner and in close collaboration with the social sciences.

It is of fundamental importance for achieving a consensus opinion of experts and the general public that the discourse is not designed to be simply an instrument for gaining acceptance, but to be more a form for identifying common social concerns. Declarations of ethical dissent based on technically argumentative discussion must, therefore, be considered as being completely valid.

Finally, the essential objectives of the project are as follows:

- gathering experience by means of an interdisciplinary discourse in a controlled environment, which will be recorded in a report and scientifically evaluated
- introducing scientific and methodological evaluation and further development of interactive media forms for the public discussion of ethical matters (Internet discussion)
- evaluating and further developing all the discussion options used with regard to their suitability for resolving value conflicts in society

Structure of the Interdisciplinary Group

Group leader
Dr. Christof Tannert (Biologist)

Sub-group leader FZJ
Dr. Peter Wiedemann (Psychologist)*

Scientists
Dr. Susanne Reif (Biologist)
Dr. Silke Schicktanz (Philosopher)*

Graduate student
Silke Domasch (Linguist)*

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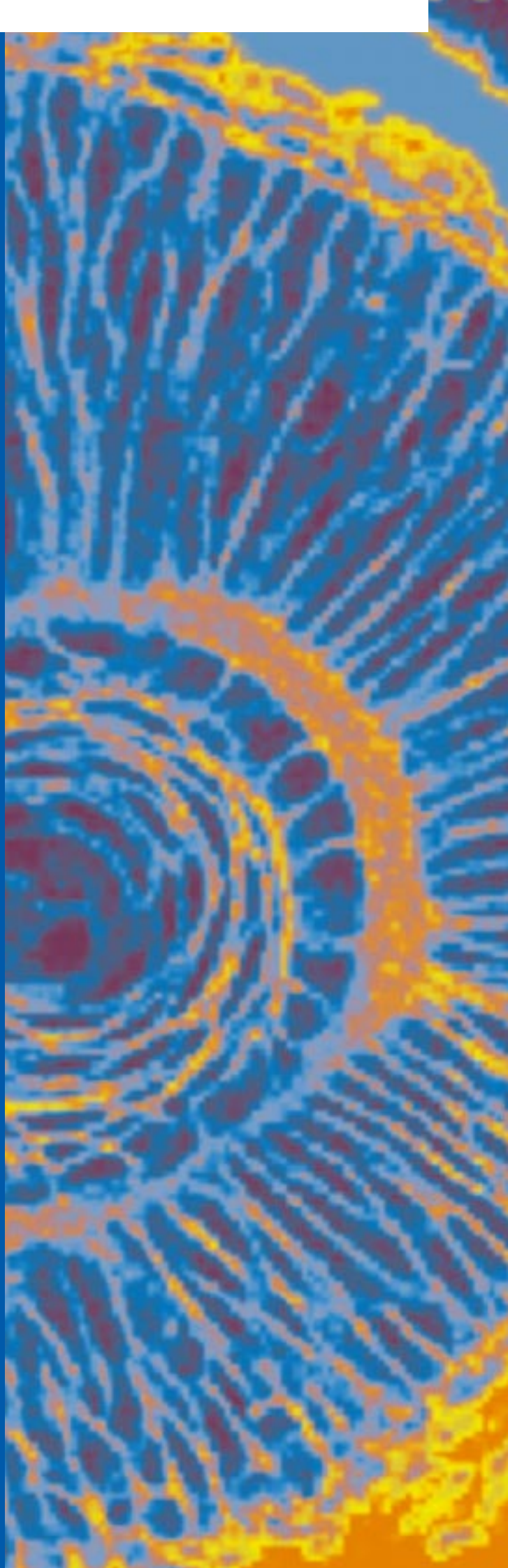
Cell Growth and Differentation



Cell Growth and Differentiation

Approximately 35,000 genes make up the human genome. Although we know the DNA sequence of all these genes, we have only a basic idea of how they are regulated and the functions they exhibit, in particular those that can induce disease. Genes encode structural and regulatory proteins that balance cell growth, differentiation and homeostasis through the control of cell-cell interactions, signal transduction, cell cycle progression, and gene regulation. In many diseases, critical genes are mutated or misexpressed so that cell growth and differentiation are distorted. Thus, it is a formidable task to identify the critical disease genes and to find out how they communicate with each other and with the rest of the genome. This is essential for developing future rational therapies for almost all diseases, including cancer and cardiovascular disease, Alzheimer disease, cystic fibrosis, or diseases affecting bone and muscle development.

In the Cell Growth and Differentiation Programme of the MDC, we study how genes and their products interact to control normal tissue development and function, and how disruption of their interactions leads to disease. A major focus of the Programme is to unravel all the features of cancer and cardiovascular disease at the molecular level using methods of molecular genetics, biochemistry and cell biology.

A reductionistic approach is often required to track the function of a gene. Accordingly, we use model systems, such as Drosophila or baker's yeast, to analyze basic gene functions. However, highly sophisticated tissue functions developed later during evolution and, so, we also need to study higher organisms that are amenable to genetic analysis such as the zebrafish or the laboratory mouse, to understand complex genetic functions. Such a combination of approaches has helped us to uncover several important mechanisms of disease development and major advances have been made in our Programme during the last two years.

For example, an important role is played by chemokines and their receptors in the process of dissemination and metastasis of solid and of haematopoietic tumors. It was found that the

Zellwachstum und Differenzierung

Das menschliche Genom umfaßt rund 35.000 Gene. Obwohl uns die DNA Sequenz aller Gene bekannt ist, wissen wir nur wenig über ihre Funktion und Regulation, insbesondere auch ihre Rolle bei der Entstehung von Krankheiten. Gene kodieren für Strukturproteine und Regulatorproteine, die Zellwachstum, Differenzierung und Homöostase durch Kontrolle von Zell-Zell-Interaktionen, Signaltransduktionen und Zellteilung in eine Balance bringen. In vielen Krankheiten sind kritische Gene mutiert oder fehlerhaft exprimiert, so dass Zellwachstum und Differenzierung gestört ablaufen. Unsere Aufgabe besteht somit darin, kritische Gene mit Krankheitswert zu ermitteln und herauszufinden, wie sie untereinander und mit dem Rest des Genoms kommunizieren. Dies ist die primäre Aufgabe, die es zu lösen gilt, um in Zukunft rationale Therapien für alle Krankheiten zu entwickeln, einschließlich Krebs, kardiovaskulären und neurodegenerativen Krankheiten, für die Zystische Fibrose oder für Krankheiten, die die Entwicklung der Knochen und Muskeln betreffen.

Im MDC-Programm für Zellwachstum und Differenzierung untersuchen wir, wie Gene und ihre Produkte miteinander interagieren, um die Entwicklung und die Funktion von normalem Gewebe zu kontrollieren, und wie Veränderungen dieser Wechselwirkung zu Krankheiten führen. Ein wesentlicher Programmpunkt besteht darin, die molekulare Basis von Krebs und kardiovaskulären Krankheiten mit den Methoden der Molekulargenetik, der Biochemie und der Zellbiologie aufzudecken.

Um der Funktion eines Gens auf die Spur zu kommen, muß man sich oft reduktionistischer Methoden bedienen. Demgemäß setzen wir einfache Modellsysteme wie z. B. die Fruchtfliege oder die Bäckerhefe ein, um grundlegende Genfunktionen zu analysieren. Jedoch haben sich komplexere Gewebefunktionen erst sehr spät im Verlauf der Evolution gebildet. Wir müssen deshalb auch höhere Organismen wie den Zebrafisch oder die Labormaus genetisch analysieren, wenn wir komplexe genetische Funktionen verstehen wollen. Durch die Kombination dieser Ansätze ist es in unserem Programm in den letzten zwei Jahren gelungen, mehrere wich-

chemokine receptors CCR7 and CXCR4 are expressed on Reed-Sternberg cells of classical Hodgkin's disease. Upregulation of CCR7 correlates with the dissemination of neoplastic cells to the interfollicular T cell zone of lymph nodes where chemokine ligands are expressed. In Hodgkin-derived cell lines, CCR7 upregulation depends on constitutive NFκB activity (Höpken et al., Blood 99, 1109-1116, 2002).

The cell adhesion and signalling protein, β-catenin, is important in embryonic development and in the formation of carcinomas. Using Cre/loxP technology, a conditional deletion of the β-catenin gene was introduced in the epidermis and hair follicles. Hair is completely lost in mutants after the first hair cycle, and regressing hair follicles are transformed into epidermal cysts. It has been demonstrated that β-catenin is essential for the ultimate fate of stem cells in the skin: in the absence of β-catenin, stem cells do not differentiate into follicular keratinocytes, but instead have an epidermal fate (Huelsken et al., Cell 105, 533-545, 2001).

Using a gene targeting approach in mice, NF-κB was shown to be required for the early morphogenesis of hair follicles, exocrine glands and teeth. The phenotype of NF-κB inhibition closely resembles hypohydrotic ectodermal dysplasia (HED) in humans and is identical to that in mice deficient in signalling molecules, called EDA or EDAR. Our data show that defective signalling through the EDA/NF-κB pathway causes HED (Schmidt-Ullrich et al., Development 128, 3843-3853, 2001).

Novel genes that are associated with metastatic tumor progression have been identified. The expression of sialyl transferase ST6Gal-II is related to poor survival of patients with colorectal carcinomas. ST6Gal-I also plays a crucial role in the adhesion and metastasis of carcinoma cells. Breast carcinoma cells that express sialyltransferase display reduced cell-cell adhesion and an enhanced invasion capacity, whereas cell clones that express an antisense-construct exhibit strong homotypic cell-cell adhesion (Schneider et al., Cancer Res. 61, 4605-4611, 2001).

C/EBP transcription factors are involved in many developmental processes that regulate proliferation and terminal differentiation in a wide variety of cell types. C/EBPα and C/EBPβ proteins occur in different isoforms that display entirely different biological activities. It has been found that regulation of translational initiation is responsible for the generation of C/EBP isoforms. Dysregulation of translation initiation pathways or misexpression of C/EBP isoforms disrupting the coupling between proliferation and differentiation programs has direct implications for cancer research, haematopoiesis, adipogenesis, lung function and female reproductive biology (Calkhoven et al., Genes & Development 14, 1920-1932, 2000).

Ubiquitin-conjugation during endoplasmic reticulum-associated degradation (ERAD) is essential for the elimination of misfolded proteins from the secretory pathway. It has been shown that the basic ERAD activity is sufficient to eliminate unfolded proteins under normal growth conditions. However, under stress, ERAD activity is increased by the UPR (Unfolded Protein Response) through transcriptional induction of

tige Mechanismen der Krankheitsentstehung aufzuklären und dabei die folgenden, spürbaren Fortschritte zu erzielen.

Eine wichtige Rolle bei der Verbreitung und Metastasierung von soliden und hämatopoietischen Tumoren spielen die Chemokine und ihre Rezeptoren. Es konnte gezeigt werden, dass die Chemokinrezeptoren CCR7 und CXCR4 von Reed-Sternberg-Zellen der klassischen Hodgkin-Krankheit exprimiert werden. Die Erhöhung von CCR7 korreliert mit der Verbreitung neoplastischer Zellen in der interfollikulären T-Zell-Zone der Lymphknoten, in denen Chemokin-Liganden exprimiert werden. In Zelllinien aus Hodgkin-Tumoren wiederum hängt die CCR7-Hochregulierung an der konstitutiven Aktivität von NF-κB (Höpken et al., Blood 99, 1109-1116, 2002).

Das Zelladhäsions- und Signalprotein β-Catenin spielt eine wichtige Rolle in der Embryonalentwicklung und bei der Bildung von Karzinomen. Mit Hilfe der Cre/lox-Technologie konnte eine konditionale Deletion des β-Catenin-Gens in die Epidermis und in die Haarfollikel der Maus eingefügt werden. Die Mutanten verlieren sämtliche Haare, und die sich rückbildenden Haarfollikel werden in epidermale Zysten transformiert. Es konnte gezeigt werden, dass β-Catenin entscheidend für das Schicksal der Stammzellen in der Haut ist: In der Abwesenheit von β-Catenin diffenzieren sich die Stammzellen nicht zu follikulären Keratinozyten und nehmen statt dessen epidermale Eigenschaften an (Huelsken et al., Cell 105, 533-545, 2001).

Mit Hilfe der Methode des „Gene-Targeting" konnte in Mäusen gezeigt werden, dass NF-κB für die frühe Morphogenese der Haarfollikel, der exokrinen Drüsen und der Zähne benötigt wird. Der Phänotyp der Inhibition von NF-κB ähnelt sehr stark der hypohydrotischen ektodermalen Dysplasie (HED) beim Menschen. Außerdem wird der gleiche Phänotyp in Mäusen beobachtet, denen die als EDA und EDAR bekannten Signalproteine fehlen. Die Daten machen deutlich, dass Signaldefekte in der Synthese von EDA/NF-κB die Ursache von HED sind (Schmidt-Ullrich et al., Development 128, 3843-3853, 2001).

Neue Gene konnten identifiziert werden, die mit Tumorprogression und Metastasierung assoziiert sind. Die Expression der Sialyl-Transferase STGal-II steht in Beziehung zu geringer Überlebensrate von Patienten mit kolorektalen Tumoren. STGal-I spielt auch eine wichtige Rolle bei der Adhäsion und Metastasierung von Karzinomzellen. Brustkarzinomzellen, die Sialyltransferase exprimieren, zeigten eine verminderte Zell-Zell-Adhäsion und eine erhöhte Invasivität, während Zellklone, die ein Antisense-Konstrukt enthielten, starke homotypische Zell-Zell-Adhäsion zeigen (Schneider et al., Cancer Res, 61, 4605-4611, 2001).

C/EBP-Transkriptionsfaktoren spielen in vielen Entwicklungsprozessen und Zelltypen bei der Regulation der Proliferation und der terminalen Differenzierung eine ausschlaggebende Rolle. C/EBP-Proteine existieren in verschiedenen Isoformen, die vollkommen unterschiedliche biologische Aktivitäten aufweisen. Es konnte gezeigt werden, dass die Regulation des Translationsbeginns für die Generierung der C/EBP-Isoformen verantwortlich ist. Fehlregulation des Translationsbeginns oder fehlerhafte Expression der C/EBP-

the involved genes. This demonstrates for the first time that a regulatory loop between ERAD and UPR is essential for the normal growth of eukaryotic cells (Friedlander et al., Nature Cell Biol. 2, 379-384, 2000).
Through large-scale screening of embryonic lethal mutations in zebrafish, the genes *heart and soul* have been identified, based on a defective heart tube morphology and a disrupted pigmented epithelium surrounding the neural retina. It has been shown that *heart and soul* encode a component of the apical Par-protein complex, termed aPKCλ. The role of aPKCλ in epithelial formation is best understood in Drosophila where Par-3, Par-6, and aPKC localize to the apico-lateral membrane of embryonic epithelia. During the early stages of epithelial differentiation, *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 morphogenesis of the heart tube and the gut with its associated organs (Horne-Badovinac et al., Current Biol. 11, 1492-1502, 2001).
Smooth muscle cells (SMC) express different isoforms of the molecular motor type-II myosin. The expression of smooth muscle MyHC (SM-MyHC) can be eliminated by gene targeting technology. Smooth muscle from knock-out neonatal mice lacked phasic contraction while tonic contraction remained normal. Accordingly, sustained force generation in the absence of SM-MyHC was sufficient for normal fetal development. However, the strong phasic contraction becomes indispensable for survival and normal growth soon after birth. In addition, both contractile systems are associated with different second messenger pathways in smooth muscle: the SM-MyHC and NM-MyHC systems appear to be involved in electromechanical and pharmacomechanical coupling, respectively (Morano et al., Nature Cell Biol. 2, 371-375, 2000).
A fundamental process in every cell division cycle is faithful duplication of genetic and epigenetic information. Construction of a chimeric green fluorescent protein fused to a central component of the DNA replication machinery (proliferating cell nuclear antigen, PCNA) permitted visualization and dynamic studies of replication sites in living cells using real-time microscopy. DNA replication in the nucleus occurs at discrete sites (replication foci) that change throughout the S-phase of the cell cycle. These changes are not due to a directional movement of replication foci but rather to asynchronous disassembly, reassembly and activation of replication at new subnuclear sites (Leonhardt et al., J. Cell Biol. 149, 271-280, 2000).

Members of our Programme have been invited speakers at many international scientific meetings during the last two years, among them Keystone Symposia, FASEB Conferences, EMBO Meetings and Workshops, ELSO Meeting, American Association of Cancer Res. Conferences, and the Banbury Meeting (Cold Spring Harbor).

The revolution in molecular biology has begun to permeate cancer and cardiovascular medicine, and molecular methods are already being used in clinical diagnosis and disease prognosis. Moreover, better understanding of cell-type specific regulatory mechanisms may eventually allow us to selectively control proliferation and differentiation processes and to find new therapeutic interventions. Potential new com-

Isoformen unterbricht die Kopplung zwischen den Proliferations- und Differenzierungsprogrammen und hat unmittelbare Implikationen für die Krebsentstehung, die Hämatopoese, die Adipogenese, die Lungenfunktion und die Ovulation (Calkhoven et al., Genes & Development 14, 1920-1932, 2000).

Für die Eliminierung falsch gefalteter sekretorischer Proteine ist die Ubiquitin-Konjugation im endoplasmatischen Reticulum (ERAD) wesentlich. Es konnte gezeigt werden, dass die grundlegende ERAD-Aktivität ausreicht, um fehlgefaltete Proteine unter normalen Wachstumsbedingungen zu eliminieren. Unter Stressbedingungen nimmt die ERAD-Aktivität durch die UPR („Unfolded Protein Response“) zu, und zwar durch Transkriptionsinduktion der betroffenen Gene. Diese Beobachtung zeigt zum ersten Mal eine regulatorische Kopplung zwischen ERAD und UPD, die für das normale Wachstum von eukaryontischen Zellen essentiell ist (Friedlander et al., Nature Cell Biol. 2, 379-384, 2000).

Durch „Screenen“ auf lethale Mutationen im Zebrafischembryo konnten die Gene *heart and soul* identifiziert werden, die mit einer defekten Morphologie des Herzens und einem zerstörten pigmentierten Epithel im Umkreis der neuralen Retina verbunden sind. Es konnte gezeigt werden, dass *heart and soul* eine Komponente des apikalen Par-Protein-Komplexes darstellt, der aPKCλ genannt wird. Eine Rolle von aPKCλ bei der Epithelbildung wird am besten in Drosophila verstanden, wo Par-3, Par-6 und aPKC an der apico-lateralen Membran embryonaler Epithelien sitzt. Zusätzlich zu den Epitheldefekten beeinflußt *heart and soul* die Morphogenese des Herzens und des Darms mit seinen assoziierten Organen (Horne-Badovinac et al., Current Biol. 11, 1492-1502, 2001).

Glatte Muskelzellen (SMC) exprimieren unterschiedliche Isoformen des molekularen Typ-II-Myosins. Die Expression von glattem Muskel-MyHC (SM-MyHC) konnte durch die Technik des „gene targeting“ ausgeschaltet werden. Glatte Muskeln in neonatalen Knock-out-Mäusen ließen die phasische Kontraktion vermissen, während die tonische Kontraktion normal blieb. Entsprechend war die nachhaltige Kraftgenerierung in Abwesenheit von SM-MyHC für die normale fetale Entwicklung ausreichend. Die starke phasische Kontraktion wird jedoch unentbehrlich für das Überleben und das normale Wachstum nach der Geburt. Zusätzlich sind beide kontraktilen Systeme mit verschiedenen „second messenger pathways“ im glatten Muskel assoziiert: Während das SM-MyHC-System mit der elektromechanischen Ankopplung verbunden ist, hat das NM-MyHC-System mit der pharmakomechanischen Ankopplung zu tun (Morano et al., Nature Cell Biol. 2, 371-375, 2000).

Ein grundlegender Vorgang bei jeder Zellteilung ist die genaue Duplikation der genetischen und der epigenetischen Information. Die Konstruktion eines chimärischen Grünfluoreszierenden Proteins, das mit einer zentralen Komponente der DNA Replikationsmaschinerie fusioniert war (dem proliferating cell nuclear antigen PCNA), erlaubte neben der direkten Beobachtung auch dynamische Studien der Replikationsorte in lebenden Zellen durch Echtzeitmikroskopie. Die Replikation der DNA beginnt in den Zellen an umgrenzten Replikationsstellen, die sich im Verlauf der S-Phase des

pounds for the treatment of diseases are presently being identified worldwide through so-called molecular screening. These procedures use molecular targets which operate in the signalling cascades of growth and differentiation processes of cells. Several groups in our Programme are involved in such research, mostly in collaboration with pharmaceutical companies. Moreover, together with the Molecular Therapy Program and the MDC clinics, gene therapy procedures are being developed based on an understanding of how genes influence growth differentiation of cells.

Walter Birchmeier
Achim Leutz

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Zellzyklus verändern. Diese Änderungen kommen nicht durch eine gerichtete Bewegung der Replikationsorte zustande, sondern durch asynchrones Zerlegen, Zusammensetzen und Aktivieren der Replikation an neuen subnuklearen Stellen (Leonhardt et al., J. Cell Biol. 149, 271-280, 2000).

In den vergangenen zwei Jahren sind Mitglieder unseres Programms zu Vorträgen auf zahlreichen internationalen wissenschaftlichen Tagungen eingeladen worden, unter anderem auf die Keystone Symposia, die FASEB-Konferenzen, EMBO-Tagungen und Workshops, ELSO-Meetings, Konferenzen der American Association of Cancer Research und zu Banbury-Meetings (in Cold Spring Harbor).

Die Revolution der Molekularbiologie durchdringt inzwischen die Krebsmedizin und die Medizin kardiovaskulärer Erkrankungen. Längst sind molekularbiologische Methoden in der klinischen Diagnostik und für prognostische Einschätzungen im Einsatz. Darüber hinaus wird uns das Verständnis der Zelltyp-spezifischen Regulationsmechanismen helfen, schließlich Prozesse der Proliferation und Differenzierung selektiv zu kontrollieren und somit neue therapeutische Eingriffsmöglichkeiten zu finden. Neue Substanzen für die Behandlung von Krankheiten werden derzeit weltweit mit Hilfe des sogenannten molekularen Screenings identifiziert. Diese Verfahren beruhen auf molekularen Zielstrukturen, die in Signalkaskaden auftreten, welche Wachstums- und Differenzierungsprozesse der Zellen steuern. Mehrere Gruppen unseres Programms sind an solchen Untersuchungen beteiligt, zumeist in Kooperation mit pharmazeutischen Unternehmen. Darüber hinaus werden mit dem Molekulare-Therapie-Programm und den Kliniken des MDC Verfahren für die Gentherapie entwickelt, die auf dem Verständnis des Einflusses der Gene auf Wachstum und Differenzierung von Zellen basieren.

Walter Birchmeier
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Growth Control and Gene Regulation in the Hematopoietic System

Achim Leutz

Cells of the blood originate from stem cells that are located in the bone marrow. Stem cells give rise to proliferating progenitors that differentiate into mature, growth arrested cell types such as, erythrocytes, granulocytes or macrophages. Dysregulation of pathways that control proliferation and differentiation may cause various diseases, such as immune defects and leukemia. Thus, hematopoiesis provides striking examples to address fundamental biological and clinically relevant questions such as: How is cell identity achieved during lineage commitment? How are cell proliferation and differentiation regulated and how are they intertwined?

Hematopoiesis and leukemogenesis are regulated by a number of key transcription factors that control developmental programs and lineage specific gene expression. Therefore, understanding the molecular basis of growth control, lineage commitment, differentiation and leukemogenesis requires the identification of regulated genes and elucidation of how these genes are regulated. Several years ago, we identified the first molecular switch that controls myeloid differentiation. The switch consists of two types of transcription factors both of which may turn into oncogenes by mutation. Transcription factors of the CCAAT/Enhancer Binding Protein family (C/EBP) regulate myeloid differentiation and proliferation arrest. The other transcription factor is derived from the Myb protooncogene and is essential for development of all hematopoietic lineages. Both types of transcription factors collaborate in a concerted action to induce myeloid gene expression. Mutations in either group of transcription factors that abrogate their collaboration may induce leukemia. This concept has now been confirmed by many research groups and has been extended to other co-operating hematopoietic transcription factors in different cell lineages. Combinatorial gene switches permit plasticity of regulation and limit the number of regulators and pathways required for cell type specification.

Chromatin remodeling and lineage specific gene expression

A prerequisite for activation of silent genes is to overcome the repressive effects of chromatin. Using an assay that monitors activation of endogenous, chromatin embedded genes, we have unraveled the mechanism of the collaboration between Myb and C/EBP. C/EBPβ interacts with the chromatin remodeling SWI/SNF complex through an N-terminal domain. This domain is contained only in one particular isoform of C/EBPβ that is generated by selective translation initiation control (see below). The interaction between SWI/SNF and C/EBP is required to activate several myeloid genes together with Myb. Grafting the N-terminus of C/EBPβ onto Myb generates a chimeric transcription factor that no longer requires C/EBPβ because it may recruit SWI/SNF all on its own. This was the first demonstration that transcription factors recruit the SWI/SNF complex to remodel chromatin during cell type specific gene expression in vertebrates. As C/EBPs participate in many cell specification events recruitment of SWI/SNF may represent a major determinant of cell lineage commitment.

Translational regulation of C/EBP

Individual C/EBP isoforms arise by differential initiation of translation. Individual isoforms mediate different functions. Full length C/EBP proteins are transactivators and inhibit proliferation while internally initiated, truncated proteins are repressors of gene activation that permit or even induce growth. Hence, regulation of translation initiation that determines the C/EBP isoform ratio plays a crucial role in the control of proliferation and differentiation.

Site directed mutagenesis has shown that a small upstream open reading frame (uORF) mediates translation control of C/EBPs. Translation regulation through the uORF is downstream of the signal transduction pathways that modulate the activity of translation initiation factors and thus the generation of C/EBP isoforms. We found that differentially initiated C/EBP isoforms display striking differences in the recruitment of chromatin remodeling complexes and in transactivation. In addition, truncated C/EBP isoforms abrogated growth arrest and uncouple proliferation and differentiation.

Differential selection of translation initiation sites is a conserved mechanism of gene expression control that allows cells to rapidly adjust to environmental, nutritional or hormonal changes. Proteins involved in translational regulation, such as PI3 kinase, AKT-kinase, PTEN-phosphatase or the initiation factors eIF-2α or eIF-4E, can also exhibit oncogenic properties. This suggests an important role of translation initiation control in cell proliferation, cell type specification and tumorigenesis. It is anticipated that pathways and factors involved in the control of translational initiation are important regulators of hematopoiesis and may represent novel targets for innovative drug therapies. Accordingly, we are searching for connections between dysregulated translation initiation control and tumorigenesis and we are developing screening systems to discover interfering drugs.

Activation of the notch pathway by E1A oncoprotein

Signaling through the Notch pathway controls cell proliferation and differentiation in many metazoan cell lineages. Following binding of its ligands, the intracellular part of the cell surface Notch1 receptor (Notch1-IC) is released and translocates to the nucleus where it alters the function of the DNA binding transcription factor CBF1/RBP-Jκ. As a result, CBF1/RBP-Jκ is converted from a repressor to an activator of gene transcription that controls expression of cell fate genes.

The Notch-CBF1 growth control pathway is exploited by the Epstein-Barr viral EBNA2 oncoprotein for viral replication. EBNA2 activates cellular and viral genes through CBF1/RBP-Jκ sites. The fact that functional CBF1/RBP-Jκ binding sites have been identified in various adenoviral promoters let us explore whether the adenoviral E1A proteins may also target CBF1/RBP-Jκ. We found that the adenoviral onco-protein 13SE1A binds to CBF1/RBP-Jκ, displaces associated co-repressor complexes, and activates CBF1/RBP-Jκ-dependent gene expression. Our results suggest that the central role of the Notch-CBF1/RBP-Jκ signaling pathway in cell fate decisions renders it susceptible to various pathways of viral replication and oncogenic conversion. Moreover, our results suggest a central role of the transcription factor CBF1/RBP-Jκ that, like the Rb and p53 tumor-suppressors, is modulated by various oncoproteins of DNA tumor viruses.

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Signal Transduction in Tumor Cells

Claus Scheidereit

Cell growth and differentiation largely depends on the expression of sets of genes which are controlled essentially at the level of transcription. Gene expression patterns are programmed by transcription factors, whose activity in turn is modulated by complex networks of signal transducing molecules. The main aim 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-κB) 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-κB activity

The pleiotropic transcription regulator nuclear factor kappaB (NF-κB) 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-κB is retained in the cytoplasm by association with IκB molecules, which inhibit nuclear translocation and DNA binding activity of NF-κB. Stimulation of cells with a variety of agents, such as bacterial lipopolysaccharides, tumor necrosis factor α (TNFα), or interleukin-1β (IL-1β) results in the proteolysis of IκB molecules and liberation of active NF-κB into the nucleus. Induced IκB proteolysis is triggered by IκB phosphorylation mediated by an IκB kinase (IKK) complex, which is activated by many NF-κB-stimulating pathways. The composition and regulation of the IKK complex is under investigation.

Differential regulation of NF-κB activity by IκBα, IκBβ, p105 and Bcl-3

The mammalian NF-κB family consists of five members, p50, p65, p52, c-Rel and RelB. These proteins form hetero- and homodimers and are bound by IκB molecules IκBα, β and ε, the precursor proteins for p50 and p52, p105 and p100, respectively, or by the nuclear IκB homologue Bcl-3.

The IKK complex phosphorylates IκBα, 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. The signal response domain of IκBα, when fused to other proteins, triggers degradation of these proteins when cells are activated by TNFα or other agents which activate IKKs. We have also found that the NF-κB precursor proteins, p105 and p100, which on processing give rise to p50 and p52, sequester other NF-κB subunits including their processing products in the cytoplasm and so act like IκB molecules. On stimulation with NF-κB activating agents, cellular p105 is phosphorylated by IKKs at two serines close to the carboxyterminal end of p105. 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 βTrCP, resulting in polyubiquitination and complete degradation by the proteasome. p105-associated NF-κB subunits, such as p50, which is formed by processing of p105, are liberated and transported to the nucleus. Thus, in parallel with the release of NF-κB dimers by IKK-induced degradation of IκBα, β or ε, other NF-κB subunits, including p50 homodimers, are released by p105 degradation. In the nucleus, p50 homodimers then associate with Bcl-3 and act as transcriptional activators.

Requirement of NF-κB for embryonic development of epidermal appendices and for secondary lymphoid organ formation

Gene ablation studies of NF-κB transcription factors and IκB kinases (IKK) have shown essential functions of these proteins for immune response and inflammation, but also for bone-morphogenesis and keratinocyte differentiation. Other functions were inaccessible due to the embryonic lethality of NF-κB or IKK-deficient animals. With a conditional gene targeting approach, we have ubiquitously expressed an NF-κB superrepressor IκBαΔN to investigate NF-κB functions in the adult. Mice with suppressed NF-κB survive to adulthood and display macrophage dysfunction and alymphoplasia, the lack of secondary lymphoid organs. NF-κB inhibition causes severe defects in the early developmental steps of epidermal appendages, including hair follicles, tear and sweat glands. Normally, these structures display strong NF-κB transcriptional activity, as we demonstrated with β-galactosidase reporter mice. This includes the stem cell-containing bulge region in hair follicles, which responds to morphogenic signals for hair follicle generation. The epidermal phenotype is analogous to hypohidrotic (anhydrotic) 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. Therefore, our data indicate that NF-κB is required in epidermal development for *edar* to transmit *eda* signals.

Requirement of NF-κB for cell proliferation and survival of lymphoma and leukemia cells

In collaboration with the research group of B. Dörken, we have discovered an essential role of aberrant constitutive NF-κB activity in the viability of Hodgkin's disease (HD) tumor cells. The NF-κB/IκB system is dysregulated in a cell-autonomous manner, involving both mutations of IκB genes and persistent activation of the IKK complex. Constitutive NF-κB blocks apoptosis and promotes proliferation and tumorigenicity of the malignant cells. A similar constitutive NF-κB and IKK activation has been found in acute lymphoblastic leukemia, and also occurs in subsets of various cancers. We have investigated NF-κB target genes in HD cells by using high density cDNA membranes and DNA chip technology. NF-κB accounts for the high expression of a gene network encoding the cell-cycle regulatory protein cyclin D2, the antiapoptotic proteins Bfl-1/A1, c-IAP2, TRAF1, and Bcl-x(L), and other pathogenetically significant molecules. Many of the identified NF-κB-dependent genes are known to exhibit frequent, marker-like expression in primary HD cells, which underscores the crucial role of NF-κB in lymphoma etiology. Future studies aim at identifying the mechanism of constitutive NF-κB and IKK activation.

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Differentiation and Growth Control in Lymphocyte Development and Immunopathogenesis

Martin Lipp

The identification and functional analysis of differentiation and growth control genes in lymphocyte development will improve our understanding how these genes are involved in the multistep process of tumorigenesis and immunopathogenesis. Many of these genes may also represent potential targets for novel therapeutical strategies. In this context, our investigations are focussing on the following research topics: i) role of chemokines and chemokine receptors in lymphocyte migration, lymphoid organogenesis and systemic immune responses; ii) immune modulatory and growth-inducing functions of chemokine receptors encoded by human herpesviruses; iii) role of lysosphingophospholipid receptors in the immune system; iv) regulation and function of CD155/polio virus receptor; v) cell cycle-dependent control of transcription.

Organization of secondary lymphoid organs by CCR7 and CXCR5 - Lessons from knock-out mice

The adaptive immune response relies on a precise temporal and spatial positioning of lymphocytes within lymphoid and nonlymphoid tissues. Chemokines, either constitutively expressed or induced during inflammation, provide a flexible navigation system directing lymphocytes into specific microenvironments. Chemokines are small basic proteins, which exert their chemoattractive activities via binding to seven-transmembrane-domain receptors signalling via heterotrimeric G proteins. Precision and specificity in this process are achieved by varying the patterns of chemokine receptors expressed on the cell surface of lymphocytes in the course of cell differentiation. The chemokine receptors CXCR5 and CCR7 are principal regulators for targeting T cells, B cells and dendritic cells into secondary lymphoid organs, thereby promoting the encounter of cells that need to interact to generate an effective immune response. The analyses of knock-out mice have been instrumental in exploring the crucial role of these receptors for the compartmentalization of secondary lymphoid organs into functionally separated T and B cell zones.

The impaired migration of lymphocytes and dendritic cells in CCR7 knockout mice causes a profound disorganization of secondary lymphoid tissues and a significant delay in mounting antibody responses and primary T cell responses. Interestingly, the recently recognized function of the lymphotoxin (LT)/TNF family and their receptors on regular lymphoid organ architecture can be attributed, at least in part, to their ability to induce expression of chemokines, CXCL13 (BLC) as well as CCL21 (SLC) and CCL19 (ELC), the ligands for chemokine receptors CXCR5 and CCR7, respectively. In cooperation with J. Cyster, UCSF, we have found that BLC-deficient mice exhibit a similar phenotype to the previously described CXCR5-deficient mice, and both molecules are required for the development of most lymph nodes and Peyer`s patches. In addition, BLC induces B cells to upregulate membrane lymphotoxin LTα1β2, which itself promotes BLC expression in follicular dendritic cells, establishing a positive feedback loop that is likely to be important in follicle development and homeostasis. This observation suggests the involvement of homeostatic chemokines in the formation of the organized ectopic lymphoid structures observed in cases of gastric lymphoma, rheumatoid arthritis, and autoimmune diseases.

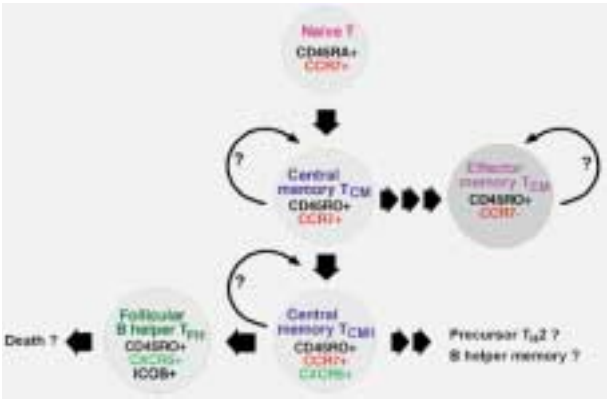
CCR7 in Hodgkin's disease

(in cooperation with H.D. Foss and H. Stein, FU; M. Hinz, MDC)

An important role has recently emerged for chemokines and their receptors involving their participation in the process of dissemination and metastasis of solid and haematopoietic tumor cells. Hodgkin's disease (HD) consists of a group of lymphomas characterized by the presence of neoplastic Reed-Sternberg cells and their variant forms most commonly located within lymphoid tissue. We have shown that chemokine receptors CCR7 and CXCR4 are expressed consistently on Reed-Sternberg cells of classic Hodgkin disease (cHD) and upregulation of CCR7 correlates with the dissemination of neoplastic cells to the interfollicular T cell zone of lymph nodes where both chemokine ligands for CCR7, namely CCL19 and CCL21, have been shown to be expressed. Interestingly, in Hodgkin-derived cell lines, CCR7 upregulation is maintained and depends on constitutive NFκB activity. In contrast to classic HD, in the so called lymphocyte predominant form of Hodgkin disease located within the B cell rich follicular structures of lymph nodes, the Hodgkin cells lack CCR7 expression.

Systemic immunoregulatory functions of homeostatic chemokine receptors

Based on the expression of human chemokine receptors it has been possible to define functionally separable memory/effector T cell populations. Previously, we have shown that the memory response is mediated by two distinct CD4+ T cell subsets: CCR7-negative effector memory T cells (T_{EM}) able to migrate to inflamed tissue, and CCR7-positive central memory T cells (T_{CM}) with the potential to home into lymphoid ogans. Recently, we have identified a novel subpopulation within the CCR7-positive central memory T cell pool that co-



Chemokine receptor-encoded differentiation of CD4+ T helper cells into functionally distinct subpopulations. Characteristic patterns of cell surface markers of the T helper subsets are indicated. Some cell populations may also be capable of self-renewal.

express CXCR5. This T cell subset, which downmodulates CCR7 upon activation, can localize via CXCR5 to B cell follicles and supports immunoglobulin (IgG, IgM and IgA) production; they have, therefore, be named follicular B-helper T cells (T_{FH}).

Immune modulatory and growth-inducing functions of viral chemokine receptors

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

We have previously shown that Epstein-Barr-Virus (EBV) specifically transactivate expression of the cellular chemokine receptor CCR7 by its regulatory nuclear factor EBNA2. In contrast to EBV, several other human herpes viruses, like cytomegalovirus (CMV) or the lymphotropic human herpes viruses type 6 (HHV-6) and Kaposi's sarcoma-associated herpes virus (KSHV), also termed HHV-8, encode viral chemokine receptors and chemokines in their genome suggesting that herpes viruses use the chemokine system to interfere with the growth and differentiation program of the host and subvert specific immune responses. Infection with KSHV has been linked by epidemiological and molecular evidence to the pathogenesis of all forms of Kaposi's sarcoma, a non-Hodgkin's B cell lymphoma, and multicentric Castleman's disease (MCD). This research project aims to establish whether the KSHV-encoded chemokine receptor (KSHV-GPCR), which is known to be constitutively activated and able to induce proliferation, plays a role in the development of human herpes virus 8-associated diseases and malignancies as an essential oncogenic or paracrine factor, or both. Generation of monoclonal antibodies specific for KSHV-GPCR revealed high expression of the viral chemokine receptor in all virus-associated diseases. Murine tumor models and KSHV-specific vaccines based on recombinant vaccinia viruses have been developed to prove whether the viral chemokine receptor induces an effective immune response.

Function and signalling of CMV encoded US28

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

The CMV encoded receptor US28 may play an important role in the pathogenesis of herpes virus infections through binding and sequestering of extracellular β-chemokines. This project is aimed at the elucidation of US28 signalling pathways underlying chemotaxis and chemokinesis in CMV infected cells. US28 displays constitutive activation of both phospholipase C and NF-kappaB signaling and its surface expression undergoes constitutive ligand-independent endocytosis. We have shown that US28 exhibits a high basal level of phosphorylation independently of ligand binding, whereas downstream phosphorylation kinetics of different mitogen-activated protein kinases is discernable upon RANTES stimulation. Currently, we have been investigating the relevance of the observed signalling events for the high internalization and recycling rate of US28 and its ability to mediate cell migration.

Role of lysosphingophospholipid 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). Although their *in vivo* functions remain largely unknown, *in vitro* extracellular application of LPA and S1P induces distinct EDG receptor-dependent cellular responses including proliferation, differentiation and migration. Our laboratory has identified and characterized EDG6, which is expressed specifically in cells and tissues of the lymphoid system. In collaboration with S. Spiegel, Washington, we have shown that EDG6 is a high affinity receptor for S1P. Recently, we have analyzed signalling pathways mediated via EDG6 and shown that EDG6 couples directly to $G\alpha_i$ and very potently to $G\alpha_{12/13}$-subunits of trimeric G-proteins. Consequently, EDG6 induces pertussis toxin-sensitive PLC activation and Rho-GTPase-dependent cytoskeletal rearrangements like peripheral stress fiber formation and cell rounding upon S1P stimulation. The capacity of EDG receptors to mediate fundamental responses such as cell motility and shape change, via $G\alpha_i$- and $G\alpha_{12/13}$-coupled signalling pathways suggests an important *in vivo* role for the S1P-EDG system in the control of cell migration in the context of the tissue microenvironment during injury and acute and chronic inflammatory responses.

Differentiation-specific regulation and function of CD155/poliovirus receptor

CD155, a transmembrane protein possessing an Ig-like architecture, was discovered originally due to its ability to serve as the cellular receptor for poliovirus (PV). However there is no information regarding the natural function of CD155. We have now provided evidence that CD155 binds specifically to vitronectin with a dissociation constant of 72 nM as determined by surface plasmon resonance. Based on sequence homology with the CD155 gene, three poliovirus receptor-related genes *(PRR1, PRR2,* and *PRR3)* were cloned recently. PRR proteins were reported by others to mediate homophilic

cell adhesion. Neither PRR1 nor PRR2 binds poliovirus and it is assumed that their physiological functions differ from that of CD155. Indeed, we found that mPRR2 binds to vitronectin only weakly, but exhibits significant self-adhesion activity, whereas there is no evidence for CD155 self-adhesion. Both CD155 and vitronectin colocalize to follicular dendritic cells and B cells inside the germinal centers of secondary lymphoid tissue (tonsils). This novel observation suggests that the CD155/vitronectin interaction might be required for the establishment of a proper immune response in this particular context.

Cell cycle-dependent transcriptional control via E2F

Cell proliferation is controlled by a network of extracellular and intracellular signalling pathways leading either to initiation and maintenance, or arrest of cell cycle progression. Transitions between certain cell cycle stages are regulated at checkpoints monitored by coordinately regulated kinase cascades turning genes on and off. Recent evidence suggests that transcription factors of the E2F-family and the tumor suppressor protein RB do not only control genes necessary for cell cycle progression, but also induce growth arrest and apoptosis upon oncogenic and hyperproliferative signals by activating p53, a tumor suppressor protein known to be phosphorylated and govern checkpoint arrest in response to DNA-damaging agents. It is further supposed that phosphorylation of p53 occurs through a DNA-dependent kinase (DNA-PK) composed of a large catalytic subunit and two DNA-targeting proteins, Ku70 and Ku80. DNA-PK is also involved in DNA double-strand break repair and recombination of immunoglobulin genes. Based on our recent finding that E2F factors physically interact via a conserved domain with Ku70 and can be phosphorylated by the DNA-PK holoenzyme, this research project proposes that functional interaction of E2F and DNA-PK abrogates E2F-dependent transcription, thereby congregating the antiproliferative and apoptotic signals induced by DNA-damaging agents.

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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. Apart from bacteria, viral systems or yeast, in metazoans both the *cis* and *trans* acting elements contributing to the initation at replication are poorly characterized. This is, however, a prerequisite for a detailed understanding of those processes controlling cellular proliferation. It would also allow new insights in the way the cell safeguards one aspect of its genomic integrity. To this end, we are investigating the architecture of chromosomal replication origins as well as analyzing the binding proteins involved. This work is being conducted with mammalian tissue cultures as well as with *Drosophila* embryos or cultured cells.

Analyzing the functional architecture of a Drosophila replication origin

The chorion gene region of *Drosophila melanogaster* encompasses an origin of DNA replication, which by now is probably the best characterized of all metazoa. Its activity has been demonstrated in specialized cells of the ovary (the follicle cells), where it directs the developmentally controlled amplification of its neighbouring chromosomal regions. In recent years we have identified and analysed the origin recognition complex (ORC) of *Drosophila.* It is expected to interact with origins in concert with other replication initiation factors. Recent data show this interaction also occurs in chorion origins. However, it remains unclear if this origin is active in cells other than follicle cells and, if so, which of its sequences directs the tissue-specific amplification program. Alternatively, the chromatin structure might play a decisive role in determining origin specificity. We plan to investigate these questions in *Drosophila* embryos as well as in insect tissue cultures. Such studies are needed to decide if the chorion replication origin can serve as a model system for origin architecture in metazoans.

Expression profiling of human replication genes

The proteins participating in the initiation of DNA replication are only poorly characterized at the biochemical level, which is largely due to the lack of a suitable *in vitro* assay for replication. As an alternative approach to gain insight into their function and regulation, we are undertaking quantitative expression profiling both at the RNA as well as the protein level. This analysis is performed by comparing cycling vs. resting and/or differentiated cells. We hope to learn which of the replication initiation factors are downregulated in nonproliferating cells and if this is actually one of the mechanisms by which unscheduled replication in these cells is prevented.

Initiation proteins as diagnostic and prognostic markers for cancer cells

in collaboration with R. C. Bargou, K. Bommert and B. Dörken, MDC

Unlike the downregulation of at least some of the replication initiation genes in differentiated cells, a reactivation of these genes has to take place in cells which reenter the cell cycle. Thus, one has to postulate that tumor cells express the complete set of DNA replication initiation genes described above, We are now analyzing quantitative and qualitative parameters of this reactivation and hope to learn if the expression of these genes can also serve as a diagnostic and prognostic marker for various malignant diseases. This part of our work relies on the immunohistochemical detection of the replication proteins. We plan to test the feasibilty of such an approach by comparing expression levels in biopsies from undiseased tissue with those from tissue representing various stages of Multiple Myeloma.

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Epithelial Differentiation, Invasion, and Metastasis

Walter Birchmeier

Our laboratory concentrates on the molecular analysis of epithelial morphogenesis and differentiation. In previous years, we have 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 loose expression of E-cadherin during tumor progression and this loss correlates with the appearance of highly invasive carcinoma cells. The function of cadherins depends directly on cytoplasmic linkage molecules, β-catenin, plakoglobin, p120, which mediate interaction of cadherins with the cytoskeleton. We have shown that β-catenin also binds to the transcription factors 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 transmission of signals from cell adhesion components and the Wnt signalling pathway to the cell nucleus.

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 recently identified a new substrate of c-met, Gab1, which mediates the signal responsible for branching morphogenesis (Weidner et al., 1996). Gab1 is a member of the family of membrane-bound multiadapter proteins which transmits signaling of tyrosine kinase receptors.

Requirement of β-catenin in anterior-posterior axis formation and hair development in mice

Jörg Hülsken, Markus Morkel, and Regina Vogel. In cooperation with Carmen Birchmeier (MDC) and Bettina Erdmann (MDC)

We recently developed β-catenin-deficient mice and observed a defect in anterior-posterior axis formation at embryonic day 5.5, as visualized by the absence of the markers Hex and Hesx1 and the mislocation of Cerberus-like and Lim1 expression. Subsequently, no mesoderm and head structures are generated. Intercellular adhesion is maintained since plakoglobin substitutes for β-catenin. Our data demonstrate that β-catenin function is essential in anterior-posterior axis formation in the mouse, and experiments with chimeric embryos show that this function is required in the embryonic ectoderm (Huelsken et al., 2000).

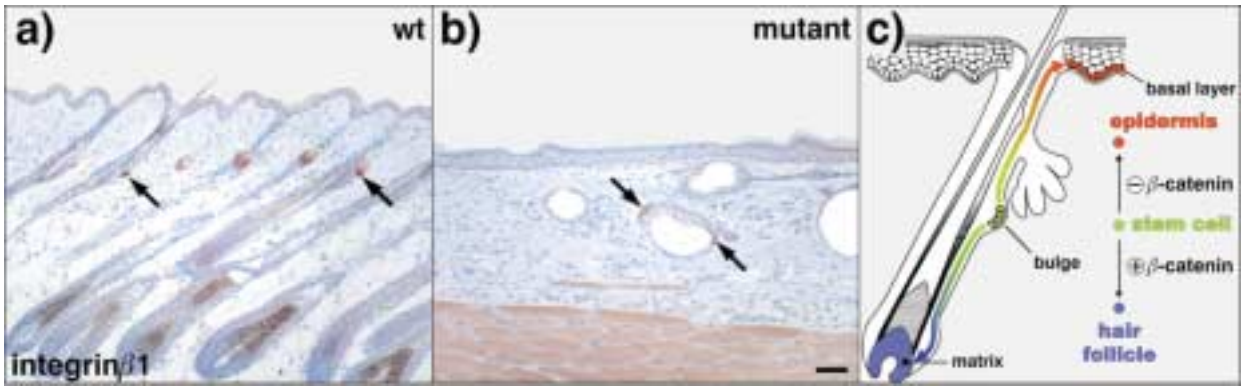
To study the role of β-catenin in development of the skin and its appendages, we introduced a conditional mutation of the gene in the epidermis and hair follicles using Cre/loxP technology. When β-catenin is mutated in the ectoderm during embryogenesis, formation of the epithelial placodes that generate hair follicles is blocked. We have shown that β-catenin is required genetically downstream of tabby/downless and upstream of bmp and shh for the formation of hair follicles. If β-catenin is mutated after hair follicles have formed, hair is completely lost after the first hair cycle, and the regressing hair follicles are transformed into epidermal cysts (see Figure). Further analysis demonstrates that β-catenin is essential for fate decisions of stem cells in the skin: in the absence of β-catenin, stem cells do not differentiate into follicular keratinocytes but, instead, adopt an epidermal fate (see Figure and Huelsken et al., 2001).

A new member of the Wnt pathway, Diversin, has been discovered and is being studied by Thomas Schwarz-Romond and Christian Asbrand. A new binding partner of tyrosine-phosphorylated E-cadherin, Hakai, has been identified by Yasuyuki Fujita. Hakai is a c-cbl-like E3 enzyme involved in turnover of E-cadherin. A gene ablation of the catenin p120 has been performed by Michael Tönjes. This results in embryonic death and defects in the axial skeleton.

Coupling of Gab1 to c-Met and downstream effectors mediate biological responses

Martin Sachs, Ute Schaeper, Dietmar Zechner, Renate Franke and Ingrid Walther. In collaboration with Henning Brohmann, Thomas Müller and Carmen Birchmeier (MDC)

The docking protein Gab1 binds phosphorylated c-Met receptor tyrosine kinase directly and mediates signals of c-Met in cell culture. Gab1 is phosphorylated by c-Met and by other receptor and non-receptor tyrosine kinases (Schaeper et al., 2000). We have performed the functional analysis of Gab1 by targeted mutagenesis in the mouse and compared the phenotypes of the Gab1 and c-Met mutations. Gab1 is essential for several steps in development: migration of myogenic precursor cells into the limb is impaired in Gab1 -/- embryos. As a consequence, extensor muscle groups of the forelimbs are virtually absent, and the flexor muscles are shorter. Fewer hind-



Differentiation of skin stem cells into the follicular keratinocyte lineage is disturbed in β-catenin-deficient skin of conditional mutant mice. (a) Stem cells reside in the bulges of hair follicles in wildtype skin. (b) Stem cells are associated with dermal cysts in β-catenin- deficient skin. Wildtype and mutant skin at day 32 after birth was stained immunohistochemically for β1-Integrin to detect skin stem cells (brown staining; arrows). (c) Scheme of the role of β-catenin in the fate decision of stem cells in the bulge: stem cells in the bulge of hair follicles can give rise to keratinocytes of both the epidermis and hair follicles. In the absence of β-catenin, skin stem cells can only form epidermis. Thus, β-catenin provides a specific signal for the differentiation of skin stem cells.

limb muscles exist, and these are smaller and disorganized. Muscles in the diaphragm, which also originate from migratory precursors, are missing. Moreover, Gab1-/- embryos die between embryonic day 13.5 and 16.5, displaying smaller livers and placental defects. The labyrinth layer, but not the spongiotrophoblast layer, of the placenta is severely reduced, resulting in impaired communication between maternal and fetal circulation. Thus, extensive similarities between the phenotypes of Gab1, c-Met and SF/HGF mutant mice exist, and the muscle migration phenotype is even more pronounced in Gab1-/-: c-Met+/- compound mutants. This is genetic evidence that Gab1 is essential for c-Met signaling in vivo. There is an analogy with signal transmission by insulin and IGF receptors, which require IRS1 and IRS2 as specific docking proteins (Sachs et al., 2000).

Novel p62dok family members, dok-4 and dok-5, are substrates of the c-Ret receptor tyrosine kinase and mediate neuronal differentiation

Jan Grimm, in collaboration with Stefan Britsch (MDC) and Kari Alitalo (University of Helsinki)

We have found that p62dok family members act as substrates for the c-Ret receptor tyrosine kinase. In addition to dok-1, dok-2, and dok-3, we have identified two new family members, dok-4, and dok-5, that can directly associate with Y1062 of c-Ret. Dok-4 and dok-5 constitute a subgroup of dok family members that is coexpressed with c-Ret in various neuronal tissues. Activated c-Ret promotes neurite outgrowth of PC12 cells; for this activity, Y1062 in c-Ret is essential. Ret/dok fusion proteins, in which Y1062 of c-Ret is deleted and replaced by the sequences of dok-4 or dok-5, induce ligand-dependent axonal outgrowth of PC12 cells, whereas a c-Ret fusion containing dok-2 sequences does not elicit this response. Dok-4 and dok-5 do not associate with rasGAP or Nck, in contrast to p62dok and dok-2. Moreover, dok-4 and dok-5 enhance c-Ret-dependent activation of mitogen-activated protein kinase. Thus, we have identified a subclass of p62dok proteins that are putative links with downstream effectors of c-Ret in neuronal differentiation (Grimm et al., 2001).

A new substrate of the c-erbB2 receptor tyrosine kinase, Vav2, has been identified by Silvana Di Cesare. Vav2 mediates alveolar morphogenesis, an important biological response of c-erbB2.

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Huelsken, J., Vogel, R., Brinkmann, V., Erdmann, B., Birchmeier, C., and Birchmeier, W. (2000). Requirement for β-catenin in anterior-posterior axis formation in mice. J. Cell Biol., 148, 567-578.

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Tumor progression, invasion and metastasis

W. Kemmner, U. Stein, W. Walther, W. Haensch, H. Schwabe, in cooperation with J. Reich and U. Karsten (MDC).

The use of novel diagnostic markers of malignancy has a strong impact on the overall survival of patients. In addition, postoperative diagnosis using markers of malignancy can enable better identification of metastatic spread to the lymph nodes and other distant sites. The human EST data base is growing rapidly with almost 3.5 million human ESTs deposited. The aim of this study was to combine this new wealth of EST expression data with detailed information collected from a colon tumor tissue bank. Using this combined data, we assayed directly sequences for their clinical relevance in terms of patient survival and or metastatic spread. To date, we have been able to identify four novel genes demonstrating a strong association with metastatic tumor progression.

Breast carcinoma cells were transfected with sialyltransferase ST6Gal-I which plays a crucial role in the adhesion and metastasis of carcinoma cells. Sense-transfected clones adhered tightly to collagen IV, showed reduced cell-cell adhesion and an enhanced invasion capacity. However, antisense-transfected clones adhered less tightly to collagen IV but showed strong homotypic cell-cell adhesion. Inhibition of ST6Gal-I by antisense-oligodeoxynucleotides could be one way of reducing the metastatic capacity of carcinoma cells.

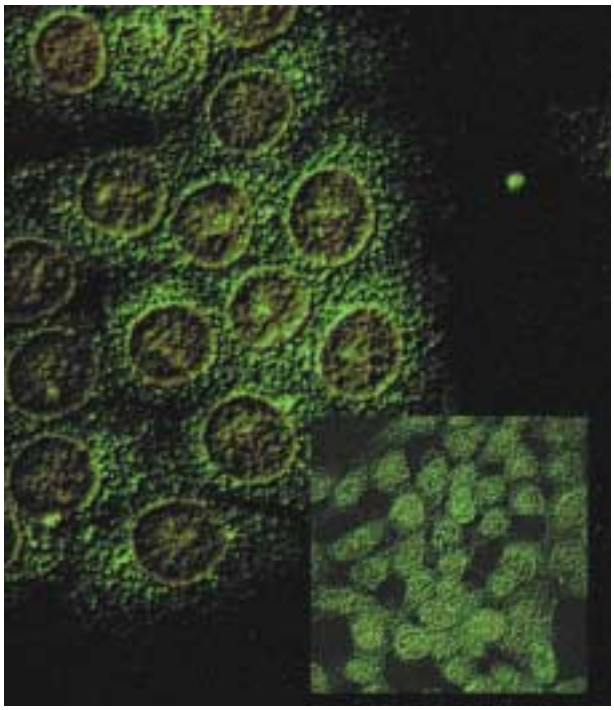
Moreover, we succeeded in characterizing a newly identified gene which is differentially expressed in human colon carcinomas and their metastases. Furthermore, this gene is overexpressed in metastasizing primary tumors compared with those which do not metastasize in any organ.
By contrast, almost no expression was detectable in normal colon mucosa. Overexpression in a human colon carcinoma cell line resulted in enhanced colony formation in soft agar, also indicating a possible function in tumor formation.

Regulation of multidrug resistance genes

Stein, U., Walther, W., Jürchott, K., Lange, C., Bergmann, S., in cooperation with H.D. Royer (MDC and University of Düsseldorf)

Drug resistance of human tumors to a variety of cytostatic agents is the limiting factor for successful cancer chemotherapy. Knowledge about the expression regulation of drug resistance-associated genes is therefore needed to circumvent or overcome the resistance phenotype. Cancer therapy-related factors, such as drugs or hyperthermia, might lead to an induction of resistance gene via stress responsive elements within the respective gene promoters. We determined expression profiles of resistance genes in human tumors, and analyzed hyperthermia-induced signal transduction pathways, focussing on the impact of the transcription factor YB-1. In human colon carcinoma cells, we found hyperthermia-induced YB-1 translocation from the cytoplasma into the nucleus, paralleled by an enhanced binding to promoters of resistance genes. Hyperthermia-induced YB-1 translocation led to an induction of expression of the drug resistance–associated genes MDR1 and MRP1. Furthermore, the expression of the resistance–associated gene MVP/LRP (lung resistance gene/major vault protein) is induced in human tumors by therapy-related modalities. Therefore, we identified the gene promoter region of the MVP/LRP gene. We isolated and characterized a 1.9 kb 5‘-flanking upstream region of this gene from a human tumor cell line, identified several consensus elements for binding of transcriptions factors, and showed the basal promoter activity of this sequence. An entire panel of promoter deletion variants is currently under investigation.

Confocal laser scanning microscopy images of the nuclear translocation of the transcription factor YB-1 in the human colon carcinoma cell line HCT116 prior to (main Figure) and post (insert) hyperthermia. Hyperthermia was performed at 43°C for 2 hours. Cells were stained using a peptide-specific polyclonal antibody against YB-1 (green) and a monoclonal antibody against lamin A/C (red).



Jet-Injection for nonviral gene therapy of cancer

W. Walther, U. Stein, in cooperation with I. Fichtner (MDC)

The novel technology of jet-injection can be applied to nonviral in vivo gene transfer. Jet-injection is efficient in gene transfer of LacZ and GFP gene constructs into different mouse tumor models and xenotransplanted human tumor models. Qualitative and quantitative expression analysis of jet-injected tumor tissues revealed efficient expression of these genes associated with a broad distribution of the transgenes in the targeted tissues. The experiments demonstrated that two or more gene constructs can be simultaneously transduced into one tissue. This nonviral gene transfer technology will be used for the transduction of "therapy-inducible" vector constructs. These vectors utilize conditionally active promoters, which can be induced by cytostatic drugs or by hyperthermia – therapeutic modalities that are used for the treatment of cancer. We have used the mdr1 promoter for such inducible expression and showed the drug- and heat-inducibility of this promoter. Further studies will show, whether nonviral gene transfer of "therapy-inducible" vectors are effective.

Pathobiology of colorectal cancers with microsatellite instability

K. Kölble, B. Barthel in cooperation with S. Scherneck (MDC)

In contrast to the majority of human colorectal cancers (CRC) which mostly derive from polyploid adenomas, approximately 10% of CRC characteristically carry somatic mutations in simple repetitive sequences. This microsatellite instability (MSI) is indicative of germline and/or somatic alterations in various DNA mismatch repair genes which, in hereditary non-polyposis colorectal cancer (HNPCC) lead to colorectal carcinogenesis in patients under the age of 50. In order to elucidate the pathogenesis of this cancer susceptibility syndrome and improve its clinical management, solid tumors of potential HNPCC cases have been registered and investigated by a combination of clinical and pedigree studies, histopathology, immunohistology, MSI and sequence analysis. Using this integrated approach, it was shown that immunohistology alone allows one to identify patients with germline mutations in the DNA mismatch repair gene MSH2 and many cases with germline mutations in the DNA mismatch repair gene MLH1. However, as some missense and truncating mutations are missed, immunohistology cannot replace testing for MSI to predict HNPCC carrier status or identify MSI-positive sporadic colorectal cancer. In addition, mutational targets specific for high grade MSI have been found in the beta-catenin (CTNNB1) and AXIN2 genes, suggesting that somatic genomic alterations in WNT-pathway genes are common to both subsets of CRC.

Mechanisms of endogenous porphyrin accumulation in colorectal carcinomas and metastases

T. Moesta, T. Handke, W. Kemmner, in cooperation with H. Rinneberg (Physikalisch-Technische Bundesanstalt Berlin)

Colorectal cancers and their metastases endogenously accumulate Protoporphyrin IX (PpIX), the immediate heme-precursor substance and a natural fluorophore. We have investigated tumor-specific alterations in the heme synthesis pathway on mRNA- and protein levels to elucidate the mechanism of PpIX accumulation in tissue samples of gastrointestinal cancers and corresponding normal tissues. Currently, surgical specimens are spectroscopically characterized by a time-delayed fluorescence spectroscopy system developed by our collaborator. To investigate subcellular localization of PpIX, a time-delayed laser-induced fluorescence microscopy procedure has been developed.

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Molecular Muscle Physiology

A European Marie Curie Training Site

Ingo L. Morano

Contractility of cardiac and smooth muscle is regulated by calcium ions (Ca^{2+}), which enter the cells through voltage-gated L-type Ca^{2+} channels and subsequently induce the release of high amounts of Ca^{2+} from the sarcoplasmic reticulum into the myoplasm through Calcium Release Channels (Ryanodin Receptors). Ca^{2+} activate intracellular signalling pathways and contraction. In cardiomyocytes, Ca^{2+} activate the contractile apparatus by binding to troponin C, which turns the thin filament from an "off" to 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 which activate the myosin light chain kinase, an enzyme which phosphorylates a 20kDa regulatory light chain of myosin, thus allowing the smooth muscle molecular motors to generate contraction on interacting 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, Ry-anodine 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

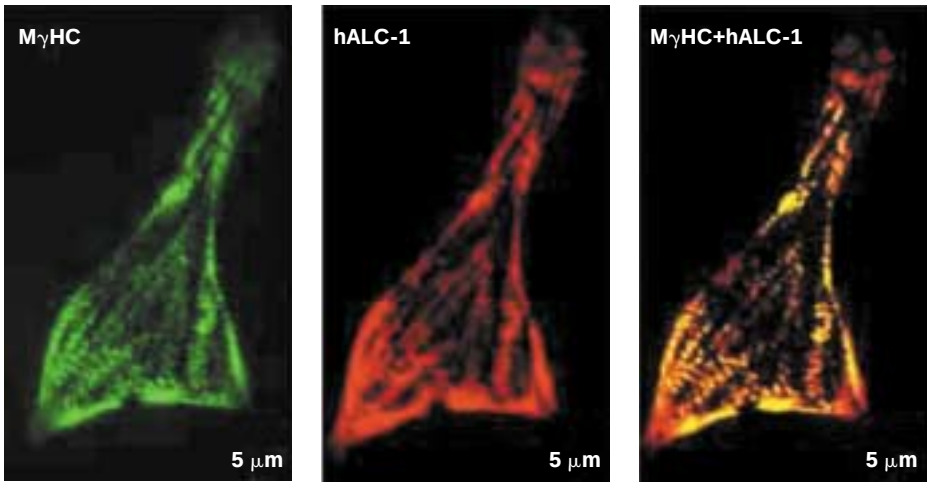
Type II myosin isoenzymes are hexamers of about 500 kDa composed of two heavy chains (MyHC) and 4 light chains (MLC), designated essential and regulatory MLC. Two genes coding for MyHC are expressed in the heart, β-MyHC and α-MyHC, located in tandem on chromosome 14. Atrium- and ventricle-specific essential (ALC-1 and VLC-1, respectively) and regulatory (ALC-2 and VLC-2, respectively) MLC exist. Cardiomyocytes of hypertrophied ventricles of patients with congenital heart diseases and hypertrophic cardiomyopathy reexpressed ALC-1, while MyHC isoenzymes did not change. This is in sharp contrast to the hypertrophied rodent ventricle which changes MyHC isoforms rather than MLC expression. Expression of the cardiac-specific basic-helix-loop-helix transcription factors eHAND and dHAND which bind to E-box elements in the ALC-1 promoter was increased in the hypertro-phied human ventricle. Ventricular cross-bridges 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-Ca2+ ratio were enhanced. The failing ventricles of patients with dilated cardiomypathy, however, hardly expressed ALC-1. Therefore, an adenoviral vector containing the human ALC-1 (hALC-1) expression cassete has been 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

Prolonged smooth muscle activation produces an initial phasic contraction with high sometric force development and maximal shortening velocity (Vmax), which is followed by a tonic contraction with low force generation and Vmax. We eliminated the expression of smooth muscle MyHC (SM-MyHC) by gene targeting technology. Smooth muscle from knock-out neonatal mice did not exhibit phasic contraction while tonic contraction remained normal. In both wild-type and knock-out animals there was a similar intracellular Ca^{2+} transient, i.e. an initial transient Ca^{2+} peak which subsequently declined to almost resting levels while tonic force development remained. Thus, the phasic contraction is generated by SM-MyHC recruitment while the sustained tonic contraction state can produce NM-MyHC activation. Non-muscle-MyHC-dependent sustained force generation was sufficient for normal fetal development. However, the strong phasic contraction caused by SM-MyHC, becomes indispensable for survival and normal growth soon after birth. 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 electromechanical and pharmacomechanical coupling, respectively.

Understanding calcium-handling proteins

Ca^{2+} channels are multisubunit complexes composed of the pore-forming α_1 subunit along with regulatory β and α2/δ subunits. Coordinated upregulation of Ca^{2+} channel subunit expression is observed in patients with hypertrophic, but not dilated, cardiomyopathy. Furthermore, we have identified fetal isoforms of both α_1 - and β-subunits. Ahnak, a 700-kDa protein, was detected in mammalian cardiomyocytes. It undergoes substantial protein kinase A phosphorylation and is preferentially localized in the plasma membrane of cardiomyocytes. Most of the C-terminus of ahnak strongly binds to the β2-subunit of the Ca^{2+} channels as well as to F-actin. Ahnak, therefore, may provide a structural basis for the subsarcolemmal cytoarchitecture and signal transduction in the car-



hALC-1 transfected Rat Cardiomyocytes
Double-immunostaining of cultivated primary cardiomyocytes transfected with an adenovirus containing the human ALC-1 (hALC-1) expression cassette. Green. stained with ant-myosin heavy chain; red: stained with an antibody raised against hALC-1; Yellow (merged) shows co-localisation.

diomyocyte. We are generating an ahnak-knock-out mouse model to understand the functional role of cardiac ahnak.

In addition, we are examining the effects of nitric oxide and reactive oxygen species on the structure and function of the cardiac Ryanodine Receptor. We have also characterized the Ca^{2+}-calmodulin-dependent kinase II (CaM Kinase II) isoforms in smooth muscle cells. CaM Kinase II is associated with the myofibrils and regulates the Ca^{2+} sensitivity of smooth muscle.

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Cell Biology of Cardiovascular Diseases

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Differentiation and proliferation of muscle cells

The uncontrolled proliferation of vascular smooth muscle cells (VSMC) leading to the formation of neointima (restenosis) and the lack of proliferative capacity of cardiac and skeletal striated muscle cells impeding regeneration after injury, are two major unsolved problems in cardiology and angiology. Restenosis is mainly due to the SMCs' capability of switching between contractile (differentiated) and synthetic (dedifferentiated) states, where the cells proliferate and migrate to form the neointima. We are investigating the signals and mechanisms controlling the proliferation and differentiation of VSMCs. For that purpose, we have cloned and characterized an isoform of a cytoskeletal protein (smoothelin) that is specifically expressed in VSMCs (J. Vasc. Res. 38, 120-132). We are now using smoothelin as a marker to trace the signal transduction pathway controlling the differentiation of VSMCs.

During 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 this can stimulate cell proliferation. This protein transduction method allows the simultaneous delivery of mixtures of regulatory proteins in a dose- and time-controlled fashion and it is easy to combine and apply this to other compounds.

Genome replication and epigenetic regulation

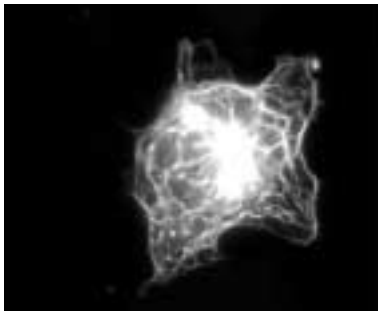
In proliferating cells, at every cell division cycle, the entire genetic and epigenetic information has to be accurately replicated once and only once. We are studying the coordination of the multiple enzymatic activities involved in this process at the cellular level. We have shown that factors involved in cell cycle regulation (cyclin A and cdk2) as well as DNA methylation (Dnmt1) are concentrated together with replication factors (RPA, DNA ligase I and PCNA) at subnuclear sites where DNA synthesis takes place during the S-phase of the cell cycle. These results indicate a high degree of coordination in space and time of different cellular processes ensuring the precise duplication of genetic and epigenetic information.

Our longterm goal is to study the architecture, assembly and regulation of these replication factories throughout the cell cycle including their interaction with cell cycle regulators and other nuclear components.To study the dynamic regulation of these nuclear structures during the cell cycle in vivo and in real time, we have developed a novel approach for the visualization of DNA replication and other nuclear structures in living cells using the green fluorescent protein (GFP). We have established cell lines expressing GFP fusions to PCNA (proliferating cell nuclear antigen, a central component of the replication machinery) and DNA ligase I. Using these cellular systems, we can show that replication foci patterns change throughout the S-phase in a characteristic manner and that the changing patterns of replication foci are not due to movements of foci through the nucleus (J. Cell Biol. 149, 271-280). Assembly and disassembly of different foci occur asynchronously suggesting that replication origins also fire asynchronously within these microscopically visible clusters. We are currently studying the kinetics of association and dissociation of different replication factors throughout the cell cycle in living mammalian cells using biophysical methods coupled with confocal microscopy.

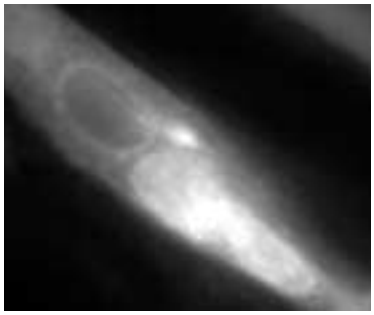
Together with the genetic information, the epigenetic information is also duplicated and maintained over many cell generations. One of the essential epigenetic modifications in mammalian genomes is the methylation of cytosines residues at position 5. DNA methylation is essential for mammalian development and has far-reaching effects on gene expression and genome structure. Our longterm goal is to elucidate the regulation of DNA methylation in mammals, in other words, how DNA methylation patterns are changed, how DNA sequences are chosen for methylation or demethylation, how fatal errors in the methylation pattern occur and how genetic and/or environmental factors might contribute to these.

We are approaching these questions via the identification and characterization of functional domains (J. Mol. Biol. 297, 293-300) of the known DNA methyltransferases (Dnmt1, 2, 3a and 3b) and the search for interacting factors which might control and direct methylation activity.
We have identified a targeting sequence in the regulatory domain of Dnmt1 that mediates the association with replication foci and might, thus, warrant the precise maintenance of methylation patterns after each round of DNA replication. The most dramatic changes in the overall DNA methylation pattern occur during preimplantation development, when

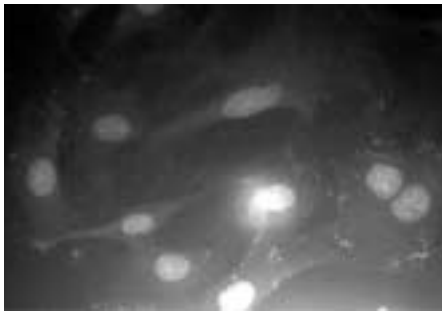
GFP-VP22
produced in Cos cells



is taken up by skeletal myotubes



and vascular smooth muscle cells



Direct Protein Delivery to Muscle Cells

most methylation patterns are erased. We have now identified and characterized a regulatory element that is responsible for the cytoplasmic localization of Dnmt1 during early development and which might, thus, cause demethylation. In addition, we have been able to identify and characterize different isoforms of Dnmt1 (Cell Growth Diff. 11, 551-559) and are now studying the role of these functional domains and isoforms in development and disease using transgenic mouse technologies.

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Intracellular Proteolysis

Thomas Sommer

The research of our group focusses on two highly conserved and essential cell biological processes: ubiquitin-proteasome-dependent protein degradation and intracellular protein transport. A link between both processes is provided by the observation that the secretory pathway of eukaryotic cells harbours an elaborate protein quality control system, which prevents deployment of the secretory pathway of misfolded or unassembled proteins. Endoplasmic Reticulum 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. Compartment-specific functions of the ubiqitin-proteasome pathway also confer specificity on the turnover of nuclear regulators. We have been able to demonstrate that the cellular localization of a protein determines its half-life, and that nucleus-restricted degradation pathways exist. Thus, selective nuclear transport provides a tool to regulate both the activity and turnover rates of nuclear regulators.

To study these processes, we are using the yeast Saccharomyces cerevisiae as an experimental system. Yeast is one of the favorite model organisms in cell biology because it can conveniently be used in both genetic and biochemical experiments.

Protein degradation at the Endoplasmic Reticulum

The basic principles of Endoplasmic Reticulum associated protein degradation have been investigated by our group. It is a process common to most eukaryotic organisms and of great medical importance. The genesis of some serious human diseases is closely linked to the ERAD system, the most prominent example being cystic fibrosis. Moreover, some human viruses use ERAD or related systems to destroy specific host proteins and manifest themselves in the infected cell.

Protein quality control requires both chaperones of the ER-lumen as well as the proteolytic ERAD pathway. The content of molecular chaperones in the ER lumen is controlled by a signaling cascade known as the unfolded protein response (UPR). We were able to obtain evidence that a coordinated regulation of UPR and ERAD system is pivotal to cell viability. This demonstrates that protein quality control in the secretory pathway is an essential cell biological process.

Based on our results we can distinguish four steps in ERAD: firstly, misfolded proteins are detected in the ER-lumen; secondly, the proteolytic substrates are transported back into the cytosol (dislocation). This requires an aqueous channel formed by the Sec61p-complex. Thirdly, dislocated substrates are marked with the polypeptide ubiquitin by the ubiquitin-conjugating enzymes Ubc1, Ubc6 and Cue1/Ubc7 and the ubiquitin ligase Hrd1/Hrd3. Fourthly, the cytosolic 26S-proteasome complex digests the misfolded proteins.

The major question is if and how transport and proteolysis are linked. We have been able to demonstrate that high-molecular weight ubiquitination of the transport substrate is a prerequisite for its dislocation. What is the function of polyubiquitination in substrate export? Our results show that it does not recruit the proteasome which, in principle, could provide the driving force for dislocation, since it also contains AAA-ATPases. So, either long polyubiquitin chains are sufficient to prevent movement back into the ER lumen, or alternatively, reverse movement may be prevented by other factors that selectively bind such chains. We favour the latter hypothesis, since we demonstrated a function of the AAA-ATPase Cdc48p in ERAD. Our data indicate that the Cdc48-complex, consisting of Cdc48, Ufd1 and Npl4, is not required for ubiquitination of substrates and does not act at the earliest stage of the pathway, but functions prior to the proteasome. It has been shown that the Cdc48-complex undergoes major structural changes following ATP-binding, which could provide a mechanical force. Moreover, it has been demonstrated that Cdc48p binds polyubiquitinated substrates. Thus, we postulate that both high molecular weight ubiquitination and the Cdc48 complex contribute to the driving force for dislocation.

The data above provide an important framework to clarify the composition of the transporter and its interplay with the ubiquitin ligation machinery.

Degradation of nuclear proteins

Recently, we could demonstrate that a nuclear substrate, the yeast transcriptional repressor Matα2, is also degraded via localized functions of the ubiquitin-proteasome system. In addition to components of the ubiquitin system, Matα2 turnover depends on signals within the protein itself. One of them is the degradation signal DEG1. We were able to distinguish two DEG1 dependent pathways: A rapid and nucleus specific one from a slow one that takes place at the cytosolic surface of the ER-membrane. Thus, a degradation signal like DEG1 is only functional in conjunction with its respective cellular localization signal, which in the case of Matα2p is the nuclear localization sequence. In cell biological terms this represents a mechanism to regulate a proteins half-life. By transporting a protein into a different cellular compartment, the turnover might be up- or down regulated, because the ubiquitination cascades are restricted to certain areas within an eukaryotic

cell. Especially for regulatory factors of the nucleus, this mechanism has been shown to be an important tool for regulation.

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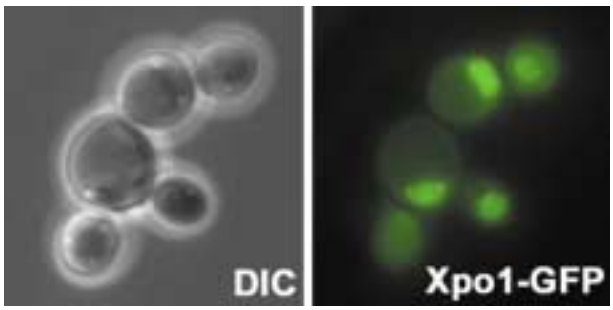
Nucleocytoplasmic Transport in the Yeast *Saccharomyces cerevisiae*

Katrin Stade (Helmholtz Fellow)

In eukaryotic cells, the nucleus is separated from the cytoplasm by a double membrane, the nuclear envelope. Nuclear pore complexes are embedded into this membrane to regulate exchange of small metabolites and macromolecules between the two compartments. Soluble transport factors, the karyopherins, travel across the pores to either import or export their respective cargo molecules. In addition, accessory factors of the so-called Ran machinery are needed to confer directionality to nuclear transport. To learn more about nuclear import and export processes, we use the yeast *Saccharomyces cerevisiae* as an experimental system. Yeast is one of the favorite model organisms in cell biology because it can conveniently be used in genetic and biochemical experiments.

Our work focusses on one particular karyopherin, the exportin Xpo1. In the nucleus, Xpo1p recognizes proteins that carry a leucine-rich nuclear export signal, the NES. It binds to its cargo protein in the presence of RanGTP and travels through the pore into the cytoplasm where the cargo is released after hydrolysis of GTP. Although, in principle, the NES-dependent nuclear export pathway is now experimentally well defined and provides an elegant working model for nuclear export in general, this process is far from being fully understood. For example, many of the cargo molecules of Xpo1p remain to be identified and whether Xpo1p is involved in the export of cellular mRNAs is still a matter of debate. Also, the role of the Yrb2 protein, which recently has been shown to be involved in NES-dependent export, is not well understood. At present, our work aims at the identification of novel NES-containing cargo molecules and a better understanding of Xpo's role in mRNA export. In addition, the functional interplay between Xpo1p and other transport factors will be investigated.

Microscopic view of living yeast cells carrying a fusion of the nuclear export receptor Xpo1p to the green fluorescent protein of *Aequoria victoria.*



Publications

Stade, K., Ford, C.S., Guthrie, C. and Weis, K. (1997) 'Exportin1 (Crm1p) Is an Essential Nuclear Export Factor' Cell 90, 1041-1050

Maurer, P., Redd, M. Solsbacher, J., Bischoff, F.R., Greiner, M., Podtelejnikov, V.P., Mann, M., Stade, K., Weis, K. and Schlenstedt, G. (2001). The Nuclear Export Receptor Xpo1p Forms Distinct Complexes with NES Transport Substrates and the Yeast Ran Binding Protein 1 (Yrb1p)' Mol. Biol. Cell, 12, 539-549

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Cytochromes P450 and Endoplasmic Reticulum

Wolf-Hagen Schunck

This group is interested in the role of arachidonic acid (AA) metabolizing P450 enzymes in the regulation of vascular tone, renal function, and the control of inflammation. In collaboration with other groups at the MDC and the Franz Volhard Clinic, we have examined pathophysiological changes in renal P450-dependent AA metabolism using mice and rat models of hypertension and end-organ damage. Moreover, we have analyzed P450-dependent epoxygenation of eicosapentaenoic acid (EPA) as a source of novel vasoactive metabolites.

P450-dependent arachidonic acid metabolism in angiotensin II-induced hypertension and end-organ damage

in collaboration with D.N. Müller, A. Mullally and F.C. Luft, Franz Volhard Clinic

These studies were performed using double transgenic rats (dTGR) which overexpress both the human renin and angiotensinogen genes. Previous studies at the Franz Volhard Clinic demonstrated that dTGR develop severe hypertension associated with impaired renal hemodynamics and tubular sodium reabsorption and die of cardiac and renal damage. We have now found that these pathologic features are accompanied by a kidney-specific down-regulation of P450-dependent AA metabolism. HPLC analysis of the metabolites produced by renal microsomal P450 enzymes revealed that both the AA epoxygenase and hydroxylase activities were significantly decreased in dTGR, compared with control rats. Moreover, the levels of P450 proteins functioning as AA epoxygenases (P450 2C and 2J isoforms) were markedly reduced. These alterations indicate an impaired capacity to produce metabolites like 20-HETE (20-hydroxyarachidonic acid) and 11,12-EET (11,12-epoxyeicosatrienoic acid). 11,12 EET and 20-HETE serve as mediators in the regulation of renal blood flow and salt excretion. Moreover, since 11,12-EET exhibits anti-inflammatory properties, the low levels of this metabolite may contribute to the onset and maintenance of angiotensin II-induced inflammation and renal damage in this rat model. Recent studies have been aimed at elucidating the mechanisms involved in the cross-talk between angiotensin II-induced pro-inflammatory and P450-mediated anti-inflammatory pathways.

P450-dependent eicosapentaenoic acid metabolites are novel activators of calcium-activated potassium (BK) channels in vascular smooth muscle cells

in collaboration with B. Lauterbach, M. Gollasch and F.C. Luft, Franz Volhard Clinic

Dietary supplementation with EPA exerts a wide range of beneficial effects on vascular function. EPA (20:5, n-3 fatty acid) is structurally closely related to AA (20:4, n-6 fatty acid) which is a source of P450-dependent vasoactive metabolites. Therefore, we first examined the question whether or not EPA can serve as an alternative substrate of AA metabolizing P450 isoforms and, second, whether or not the EPA metabolites produced have vasoactive properties.

To address the first question, we selected P450 isoforms expressed in endothelial cells (P450s 2C8 and 2J2) and in vascular smooth muscle cells (P450s 4A1, 4A2 and 4A3). Using recombinant P450 enzymes, we found that all the P450 isoforms tested are able to convert AA and EPA with similar efficiencies. Converting AA, P450s 2C8 and 2J2 produced a series of regio-isomeric EETs that are known to activate BK channels in vascular smooth muscle cells and to cause vasorelaxation. EPA was converted by these P450 isoforms to one major metabolite. We identified the metabolite as 17,18-epoxyeicosatetraenoic acid (17,18-EETeTr). Further analysis by chiral-phase HPLC revealed predominant formation of the 17(R),18(S)-enantiomer. P450s 4A1, 4A2, and 4A3 hydroxylated AA to 19- and 20-HETE, which is known to act as a vasoconstrictor by inhibiting BK channels. With EPA as substrate, the analogous hydroxylation products, 19- and 20-OH-EPA, were formed. However, P450 4A1 was also able to epoxygenate EPA and produced 17,18-EETeTr with a stereoselectivity favouring the R,S-enantiomer.

To gain insight into the potential vasoactive nature of the P450-dependent EPA metabolites, we synthesized these metabolites by chemical means and tested them for their effects on the BK channel activity. Patch-clamp studies demonstrated that 17(R),18(S)-EETeTr is a highly potent activator of BK channels in rat cerebral arterial vascular smooth muscle cells. 17(R),18(S)-EETeTr shared this property with 11, 12-EET, the compound that is proposed to function as the primary endothelium derived hyperpolarizing factor in a number of vascular beds. Direct comparison showed that EPA epoxide even exceeded the effect of the AA epoxide. Indicating a high degree of stereo- and regioselectivity, only the 17(R),18(S)-EETeTr was active but not the respective S,R-enantiomer or other regioisomeric EPA epoxides. Taken together with our results on P450 expression and activities, we propose that competition between AA and EPA for conversion by P450 enzymes and the resulting formation of alternative metabolites, such as 17(R),18(S)-EETeTr, may contribute to the beneficial effects attributed to diets rich in EPA e.g. fish oil.

Selected Publications

Gross, V., Schunck, W.-H., Honeck, H., Milia, A.F, Kärgel, E., Walther, T., Bader, M., Inagami, T., Schneider, W., Luft, F.C. (2000). Inhibition of pressure natriuresis in mice lacking the AT2 receptor. Kidney Int. 57, 191-202.

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Schwarz, D., Kisselev, P., Honeck, H., Cascorbi, I., Schunck, W.-H., Roots, I. (2001). Co-expression of human cytochrome P4501A1 (CYP1A1) variants and human NADPH-cytochrome P450 reductase in the baculovirus/insect cell system. Xenobiotica 31, 345-56.

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Schwarz, D., Kisselev, P., Cascorbi, I., Schunck, W.-H., Roots, I. (2001). Differential metabolism of benzo[a]pyrene and benzo[a]pyrene-7,8-dihydrodiol by human CYP1A1 variants. Carcinogenesis 22, 453-9.

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Cell Polarity and Epithelial Formation in Development and Disease

Salim Abdelilah-Seyfried

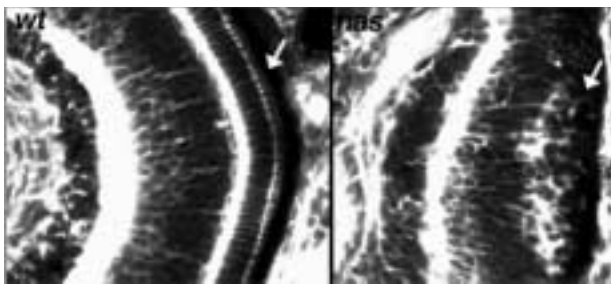
The formation and maintenance of epithelial sheets are essential for correct development and for normal physiological properties of organs and tissues. The loss of epithelial integrity in mature tissues can cause various medical conditions, including degenerative pathologies of the cardiovascular and nervous systems and tumor formation. The cellular machinery involved in establishing and maintaining cellular polarity within epithelial cells is intricately involved in epithelial morphogenesis. Mature epithelial cells are highly polarized with separate apical and baso-lateral membrane compartments, each with a unique composition of lipids and proteins. Among the known cellular components involved in generating this cellular polarity is the cadherin/catenin-based protein complex at the adherens junction and the Par-3/Par-6/atypical protein kinase C (aPKC) complex at the apico-lateral membrane. Our laboratory uses two genetic model organisms, zebrafish *(Danio rerio)* and fruitfly *(Drosophila melanogaster)*, for comparative studies of the role that these molecular pathways play during the formation of epithelial cell sheets. Previous screens in zebrafish have isolated mutations with medically relevant epithelial phenotypes. The analysis of these mutations can provide insight into vertebrate development and disease.

The Par-3/Par-6/aPKC complex in zebrafish epithelial formation and organogenesis

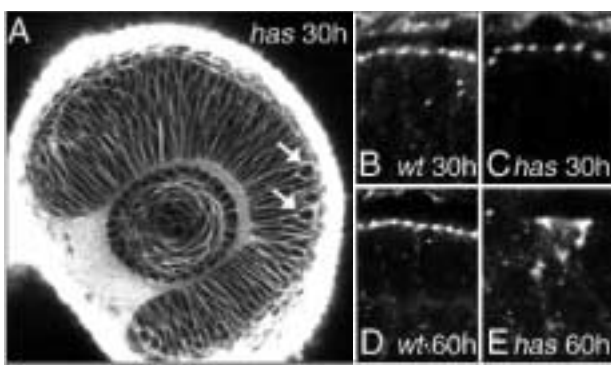
Large-scale screens for embryonic lethal mutations in zebrafish have isolated several mutations that affect epithelial integrity. One of these mutations, *heart and soul*, was identified based on a defective heart tube morphology and a disrupted pigmented epithelium surrounding the neural retina. We have shown that the zebrafish locus *heart and soul* encodes an aPKCλ, one of the two closely related isoforms of atypical PKCs found in vertebrates and a component of the apical Par-protein complex. At the core of this protein complex are the PDZ-domain containing proteins, Par-3 and Par-6, and an aPKC. 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. Loss of function of any one of these genes causes epithelial defects, including a loss of cellular polarity, loss of apical adherens junctions, and changes in cell shape.

Consistent with a conserved role of zebrafish aPKCλ, the protein localizes to the apico-lateral membrane partially overlapping adherens junctions and is required for the formation and maintenance of adherens junctions in the polarized epithelia of the retina, neural tube, and digestive tract. During the early stages of organogenesis, when epithelial phenotypes first appear, *heart and soul* seems to regulate the apical clustering and maintenance of adherens junctions. In addition to the epithelial defects, *heart and soul* affects the morphogenesis of the heart tube and the gut with its associated organs. It is unclear whether these defects are caused by underlying epithelial defects.

Retina 2 Phalloidin staining of the zebrafish retina at 72 hours. (A) In wild-type, a columnar epithelium of photoreceptor cells is present at the ventricular (apical) surface of the retina (white arrow). Cell-cell contacts are established by a belt of actin-rich adherens junctions at the apico-lateral membrane of these cells. (B) In *heart and soul* mutants, a disorganized layer of rounded cells is present at the ventricular surface (white arrow).



RetinaAJ3 The adherens junction is not maintained in *has* mutant retinae. (A) The eye of a live 30 hour *heart and soul* mutant stained with BODIPY-ceramide. The gross morphology of the pseudostratified retinal neuroepithelium is indistinguishable from wild-type at this stage, and most cell divisions occur at the ventricular (apical) surface (white arrows). (B,C) In 30 hour wild-type and *heart and soul* mutant retinae, adherens junctions are present as indicated by strong apical ZO-1 localization. (ZO-1, green; aPkC, red) (D) In the 60 hour wild-type retina, ZO-1 localization at the apical adherens junction is maintained. (ZO-1, green; β-catenin, red) (E) There is a strong reduction in apical ZO-1 positive junctions in *heart and soul* mutants at 60 hours, indicating that most apical adherens junctions are missing.



Research in our laboratory is currently directed towards 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 novel therapeutic approaches to be investigated.

Selected Publications

Abdelilah-Seyfried, S., Chan, Y.M., Zeng, C., Justice, N., Younger-Shepherd, S., Sharp, L., Barbel, S., Meadows, S., Jan, L. and Jan, Y.N. (2000). A gain-of-function screen for genes that affect the development of the *Drosophila* adult external sensory organ. Genetics, 155, 733-752.

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Electron Microscopy

Gerd Kempermann
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This facility offers a range of conventional and advanced electron microscopic methods to all interested research groups in the institute. The well-established methods of immunohisto- and cytochemical labeling have been supplemented by various plastic embedding techniques, allowing the visualization of morphological details even in complex tissues. The facility is available for all types of collaboration including advice in preparation techniques, service, and the execution of common research projects.

Phenotype analysis in normal and genetically modified organisms

Conventional electron microscopy of Eponembedded tissue allowed the following results to be obtained:

Reduction of the +KTS isoform of transcription factor WT-1 results in impaired foot process formation in podocytes and death of newborn mice because of impaired renal filtration function. (G. Lutsch in collaboration with A. Schedl, see Fig. 1)

Knock-out mice for the smooth muscle myosin heavy chain revealed thick myosin filaments in bladder tissue, indicating a role of cytoplasmic myosin in smooth muscle contraction. (G. Lutsch in collaboration with I. Morano)

Conditional mutation of the β-catenin gene in mice revealed the fundamental role of β-catenin in the fate of skin stem cells. Cells of hair follicle-derived cysts in mutants have lost their follicular characteristics and showed epidermal differentiation. (B. Erdmann in collaboration with W. Birchmeier and J. Hülsken)

In addition, the following tissue types in different mouse and zebra fish mutants were investigated: Notochord, axon bundles of the olfactory bulb, retina, peripheral nerves, heart and sceletal muscle. Morphological changes in certain cases could be supported by immunocytochemical labeling. (B. Erdmann in collaboration with C. Birchmeier, A. Garratt, W. Birchmeier and F. Rathjen)

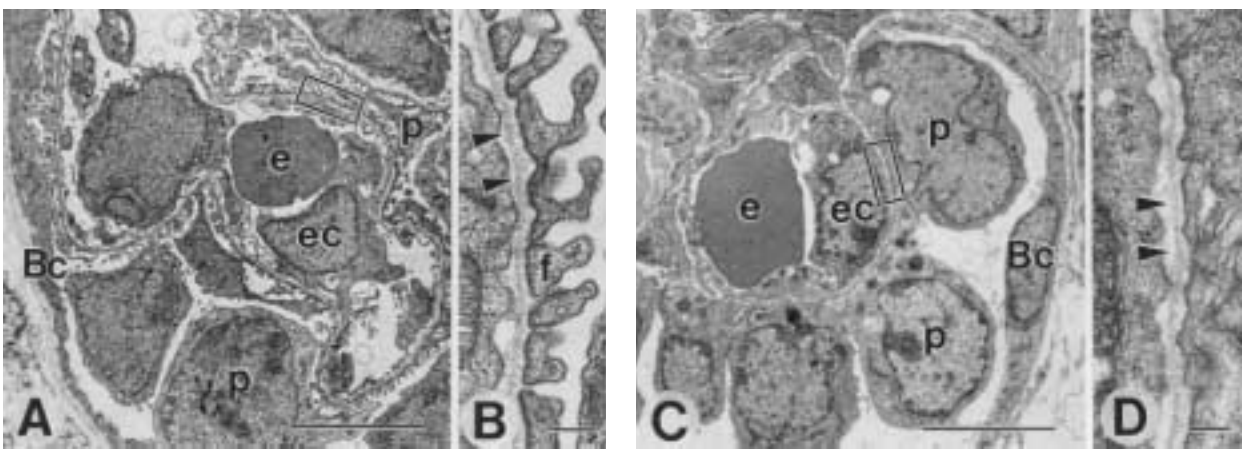
Identification of neural stem cells in murine hippocampus

A method was developed which allowed the identification of BrdU-labeled proliferating cells in the murine dentate gyrus by correlative immunofluorescence and immunoelectron microscopy. Further studies are intended to analyze the differentiation of these cells by labeling with neuron- and glia-specific markers. (G. Lutsch in collaboration with G. Kempermann)

Further collaborations:

In situ-hybridization studies in mouse kidneys to localize cytochrome P450 4a isoforms (B. Erdmann with W.-H. Schunck and V. Gross)

Electron micrographs of glomeruli of neonatal wild-type (A, B) and WT1-knock-out mice (C, D). A,C overviews; B, D enlargements of selected areas marked in A, C. Note impaired foot process formation in C, D. Bc, Bowman`s capsule; e, endothelial cell; ery, erythrocyte; f, foot process; p, podocyte. Arrowheads point to the basal membrane between endothelial cells and podocytes. Bar = μm



Electron microscopic investigations of

- Vav2-induced alveolar morphogenesis of mammary gland epithelial cells (B. Erdmann with W. Birchmeier and S. Di Cesare)
- localization of CALEB in the superior colliculus and the retina in mouse and chicken (B. Erdmann with F. Rathjen and M. Moré)
- neuron-glia interaction in the cerebellum of EGFP/GFA transgenic mice (B. Erdmann with H. Kettenmann and C. Nolte)
- detection of foreign DNA in endothelial cells following non-viral gene transfer (B. Erdmann with M. Boettger)
- location of the small heat shock proteins, Hsp 25 and _B-crystallin, in normal and ischemic rat kidney (G. Lutsch with W. Smoyer and R. Benndorf, Ann Arbor)
- identification of the sequence segments of Hsp 25 that are responsible for inhibition of actin polymerization (G. Lutsch with R. Benndorf, Ann Arbor)
- characterization of folding pathways of model proteins (G. Lutsch with G. Damaschun)

Selected Publications

Hammes, A., Guo, J.K., Lutsch, G., Leheste, J.R., Landrock, D., Ziegler, U., Gubler, M.C., and Schedl, A. (2001) Two splice variants of the Wilms`tumor 1 gene have distinct function during sex determination and nephron formation. Cell 106, 319-29

Huelsken, J., Vogel, R., Erdmann, B., Cotsarelis, G., and Birchmeier, W. (2001) β-catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell 105, 533-545

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Smoyer, W.E., Ransom, R., Harris, R.C., Welsh, M.J., Lutsch, G., and Benndorf, R. (2000) Ischemic acute renal failure induces differential expression of small heat shock proteins. J. Am. Soc. Nephrol. 11, 211-221

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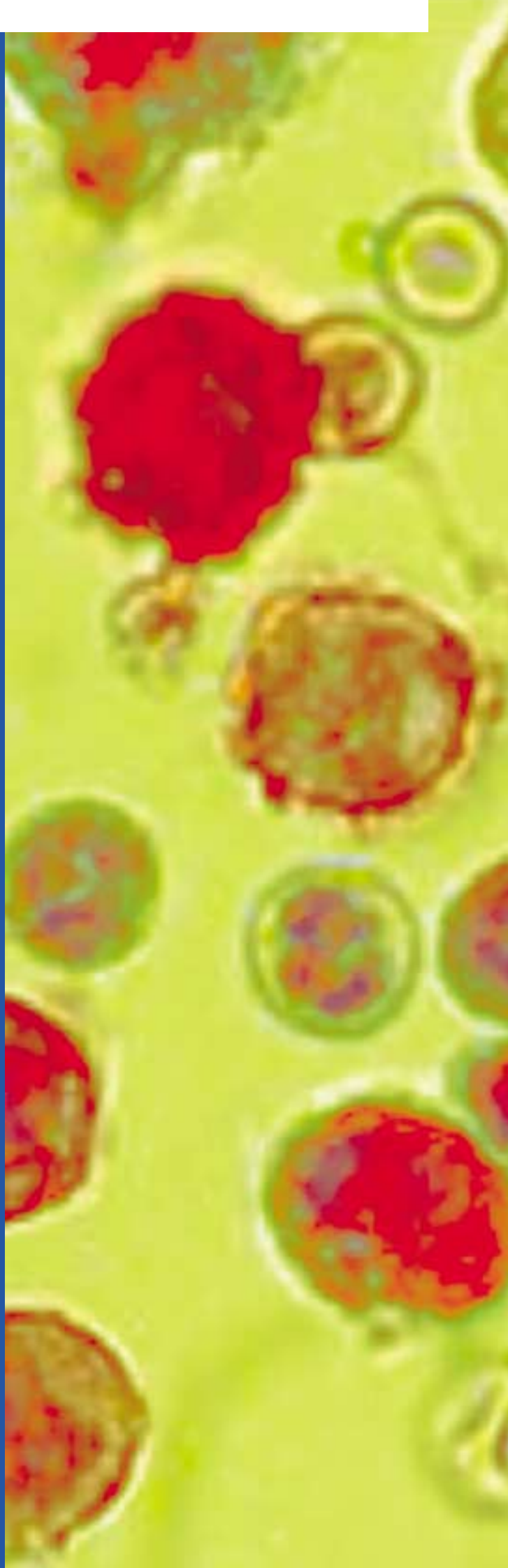
Molecular Therapy



Molecular Therapy

The aim of this program is to develop new therapeutic strategies for those diseases, such as cancer or cardiovascular disease, which often remain resistant to treatment. Our efforts are based on a wide variety of experimental strategies that exploit the latest knowledge emerging from the fast-growing fields of cell biology, cancer biology, immunology and mammalian genetics. The molecular mechanisms that underlie malignant transformation have been unravelled to a considerable degree, and a great deal is now understood about how tumors become resistant to standard therapies and escape immune recognition and destruction. For instance, it is recognized that tumors often express potentially immunogenic antigens that, nevertheless, fail to elicit an effective immune response from the host. It is also know that T cells must undergo an elaborate activation process in order to reject malignant tissues. An arsenal of cloned genes is now available whose products are involved in cell-cycle arrest, apoptosis, selective killing of tumor cells, and the induction of immune responses. Combining this knowledge and these reagents, a number of gene transfer technologies now allow the development of very precise and, hopefully, more effective and less toxic therapeutic modalities.

Molekulare Therapie

Das Ziel dieses Programms besteht darin, neue therapeutische Strategien für Erkrankungen zu entwickeln, die häufig nicht auf traditionelle Behandlungsmethoden ansprechen, wie dies bei Krebs oder Herzkreislaufkrankheiten der Fall ist. Unsere Bemühungen basieren auf einer großen Vielfalt von experimentellen Strategien, die den neuesten Stand des Wissens nutzen, wie er sich aus den rasch wachsenden Bereichen der Zellbiologie, Krebsforschung, Immunologie und der Säugetiergenetik ergibt. Die molekularen Mechanismen, die bösartigen Transformationen zugrunde liegen, konnten zu einem Großteil aufgeklärt werden, und sehr gut verstanden ist auch, wie Tumoren gegenüber Standardtherapien resistent werden und der Erkennung und Zerstörung durch das Immunsystem entgehen. So ist zum Beispiel bekannt, dass Tumoren zwar oft potentielle immunogene Antigene exprimieren, ohne dass es diesen Molekülen aber gelingt, beim Wirt eine Immunreaktion auszulösen. Es ist auch bekannt, dass T-Zellen einen ausgedehnten Prozeß der Aktivierung durchlaufen müssen, um bösartig verändertes Gewebe abzustoßen. Ein Arsenal von klonierten Genen steht heute zur Verfügung, deren Produkte zu der Arretierung des Zellzyklus, zur Apoptose, zum selektiven Absterben von Tumorzellen und der Induktion der Immunreaktion beitragen. Die Kombination dieses Wissens mit den genannten Agenzien liefert eine Vielzahl von Gentransfertechnologien, mit deren Hilfe die Entwicklung von sehr präzisen und hoffentlich auch wesentlich effektiveren und weniger toxischen therapeutischen Modalitäten entwickelt werden kann.

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 as far as regeneration is concerned. So far, conventional therapy given to patients with heart failure aims at reducing the hemodynamic load in order to alleviate cardiac function. 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 modells of primary cardiomyocytes, our group is trying 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 remain unknown. Therefore, the intercalation between pro- and anti-apoptotic pathways on one side and classical growth cascades including cell cycle pathways on the other is another focus of our research interest.

Regulation of E2F1-dependent transcription and apoptosis by the ETS-related transcription factor GABPγ1

In this regard, our observation is of interest that both cyclin-dependent kinase inhibitors, p21CIP1 and p27KIP1, need to be downregulated in order to trigger apoptosis in cardiomyocytes. Also, 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 GABPγ1. This interaction links growth and cell death related 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, has been 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 levels in adult cardiomyocytes.

While trying to understand how cell death of cardiomyocytes is triggered, much of the effort of our group is devoted to deciphering the regulation of cardiomyocyte cell cycle withdrawal. Employing different models we have found that p21-CIP1 plays a critical role in the prevention of cardiomyocyte cell cycle activation. Importantly, p21CIP appears to act as a suppressor of the 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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Control of Smooth Muscle Cell Function

Maik Gollasch (Helmholtz fellow)

Work in the laboratory lead by Maik Gollasch 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.

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 (Figure A). We use a combined approach, involving single cell isolation, ion channel recording techniques, intracellular calcium and 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 calcium influx through a single, caveolemmal calcium channel triggers calcium sparks (Figure B), Furthermore, the β1-subunit (BKβ1) of the large-conductance calcium-activated potassium (BK) channel represents the molecular sensor of calcium sparks to reduce myogenic tone. Deletion of BKβ1 disrupts coupling between calcium sparks and BK channels, leading to increased arterial tone through an increase in global intracellular [Ca^{2+}] and increased systemic blood pressure in mice. Current work is examining the role of RyR isoforms in release of sparks and BKβ1 variants in humans. The BKβ1 gene may be an important candidate gene for 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 releases an adventitium-derived relaxing factor (ADRF) that acts by activation of KATP channels. In collaboration with Dr. Wolf-Hagen Schunck's group, we have investigated the effects of P450-dependent epoxygenation of eicosapentaenoic acid on potassium channels. Current work examines the identity and role of "ADRF" in the resistance vasculature.

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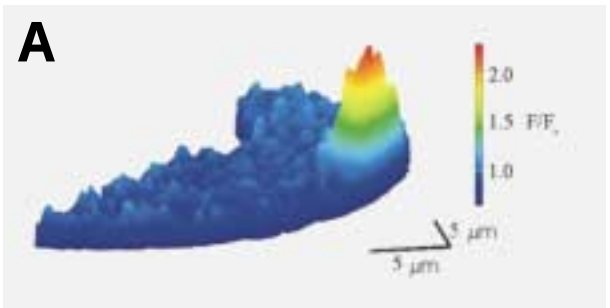
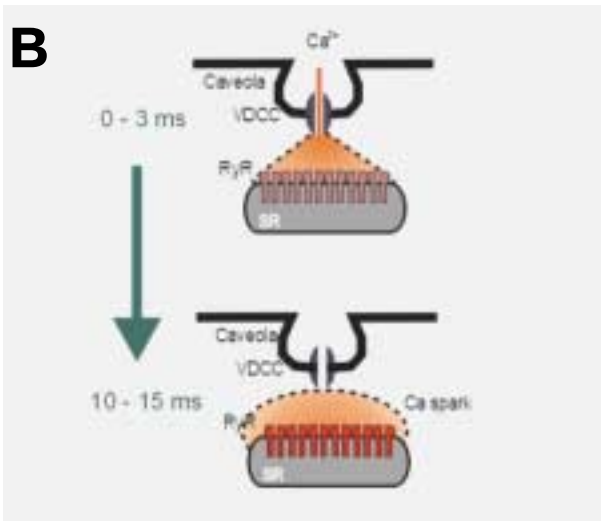
Calcium spark in a single smooth muscle cell from rat cerebral arteries (A) and ignition of calcium sparks through a single, caveolemmal voltage-dependent calcium channel (B). 3-D plot of a Ca^{2+} spark in an individual myocyte occurring in close proximity to the plasma membrane (A, from Gollasch et al., 1998). Shown is an entire-cell image recorded within 16.7 ms. The peak increase in fluorescence (F/Fo) of Ca^{2+} sparks is approximately 2.7, with a mean spatial spread on the order of 2.5 µm and a half-time of decay of approximately 50 ms.





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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 have identified functional autoantibodies against extracellular structures of G-protein coupled receptors.

We have observed autoantibodies against adrenergic receptors and AT_1-receptors in the sera of patients with myocarditis, dilated cardiomyopathy, and hypertension. These autoantibodies recognize epitopes on the first or second extracellular loop of the receptors and act as the corresponding pharmacological agonists. In patients with myocarditis and dilated cardiomyopathy, as well as in Chagas´disease, the autoantibodies recognize β1-adrenoceptors. In patients with hypertension we observed autoantibodies against the α1-adrenergic and the angiotensin II AT_1-receptor.

In recent years we have investigated in more detail the effects of these autoantibodies, and have been able to show that the antibodies stabilize the dimeric agonistic conformation of the receptors resulting in an agonist-like effect on the autoantibodies.

Autoantibodies in myocarditis and dilated cardiomyopathy

The suggestion that the anti-β_1-adrenoceptor autoantibody might 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. It is, therefore, also of interest in the present context that, in a patient with acute myocarditis, the healing process, as reflected by a normalization of the ejection fraction and the heart rate, correlates with a disappearance of anti-β_1-adrenoceptor autoantibodies from the blood.

Based on our autoimmune hypothesis, we have proposed new therapeutic options to treat patients with endstage dilated cardiomyopathy. One of them is immunoadsorption using Therasorb columns to remove immunoglobulins from the patient's plasma. After this treatment, a marked improvement in cardiac function and a normalization of the heart size was observed.
The strong correlation observed between the reduction in the amount of circulating autoantibodies against the β_1-adrenoceptors and improvement in heart function just described 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 have developed a specific immunoadsorption column. Based on our epitope analysis, a peptide column was developed that selectively removes anti-β_1-adrenoceptor autoantibodies. This column is presently being used in a clinical pilot study.

Autoantibodies in hypertension

We have also investigated the role of autoantibodies in essential and malignant hypertension. In some sera of patients with this disease we have detected autoantibodies directed against the α1-adrenergic receptor. These autoantibodies recognize epitopes of the first or second extracellular loop of the α1-adrenergic receptors and acting like α1-adrenergic agonists.

In patients with malignant hypertension and in patients with preeclampsia we have observed autoantibodies against the angiotensin II AT_1-receptor. In preeclamptic patients, this antibody is detectable after the 20^{th} week of pregnancy and disappears after delivery. The anti-AT_1-receptor antibodies act like the agonist angiotensin II and induce the formation of the transcription factor AP-1. These functional autoantibodies are found in all preeclamptic women investigated so far and may play a role in elevating vascular resistance and promoting hypertension and cardiac hypertrophy in these patients.

Role of mast cells in the heart

Another topic of our research involves cardiac mast cells. Because the heart of patients with dilated cardiomyopathy contain four times more mast cells and more histamine than controls, we wanted to identify the role of these cells. Using a monoclonal antibody against surface determinants of rat connective tissue mast cells, we have been able to identify a great number of, mostly undifferentiated, mast cells in the neonatal rat heart and in cell cultures prepared from this organ. In cell culture, we were able to differentiate the mast cells. These differentiated mast cells, mostly in intimate contact with cardiomyocytes, synthesize mediators histamine, serotonin and tumor necrosis factor α (TNFα).

In heart tissue of DCM patients, we have investigated the degranulation of the mediators TNFα and tryptase from mast cells. Both mediators may be involved in the development of fibrosis and remodelling in the failing heart.

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Bernd Dörken

Identification of genetic risk profiles and gene therapy of malignant tumors

Peter Daniel, Bernhard Gillissen, Philipp Hemmati, Isrid Sturm, Thomas Wieder

Cell cycle deregulation and apoptosis is the underlying cause of tumorigenesis and tumor progression. The inactivation of key regulators of apoptosis contributes to the development of resistance to therapy. We have shown the relevance of such events in the p53 signaling pathway. Multigene analysis of upstream regulators and downstream effectors of p53 in patients with cancer of the upper and lower gastrointestinal tract, breast cancer and acute and chronic lymphocytic leukemias shows that the loss of Bax, apart from mutation of p53, is a central event in the development of resistance. Patients with an intact Bax show, in most cases, a better response to therapy and improved survival. There are, however, exceptions to this rule, such as in gastric cancer where separate genetic events appear to be responsible for the poor prognosis in this disease. We have shown that the inclusion of additional genes such as downstream regulators of apoptosis, i.e. APAF-1, IAPs, and the caspases as well as components of the Rb pathway of cell cycle regulation improves the identification of patients with a good or poor prognosis. Apart from providing a better understanding of resistance to conventional cancer therapy, we have also laid the foundation for novel, molecular therapies. The aim is to transfer cell cycle-inhibitory and apoptosis-inducing genes into tumor cells. To this end, we have demonstrated that overexpression of procaspase-3, Bak, Bik/Nbk, Bax and p14ARF may sensitize cancer cells to conventional cytotoxic therapies or induce apoptotic cell death by itself.

Biology of Hodgkin's disease

Stephan Mathas, Martin Janz, Franziska Jundt, Kristina Schulze-Pröbsting in cooperation with Claus Scheidereit (MDC) and Harald Stein (UKBF)

Constitutive NF-κB activity is a characteristic of Hodgkin-/Reed-Sternberg (HRS) cells and is required for proliferation and survival of these cells. We have identified different defects in the signaling cascade leading to the constitutive NF-κB activity. Thus, mutated IkB proteins, detected in cell lines and lymph node biopsies, are unable to bind to NF-κB. Furthermore, the IκB kinase (IKK) complex is constitutively active in HRS cells. The central role of NF-κB in the biology of HRS cells was confirmed by the identification of NF-κB-dependent anti-apoptotic genes in HRS cells. Currently, we are investigating the possibility of establishing the inhibition of constitutive NF–κB activity as a specific treatment option for Hodgkin's disease. Different pharmacological inhibitors of transient NF-κB activation, including aspirin or arsenicals, have been described. For some of these drugs, we have been able to show strong in vitro and in vivo anti-tumor activity. Further studies have examined the role of NF-κB-related transcription factors and signaling pathways and their role in HRS cells. In addition, mechanisms leading to constitutive NF-κB activity are being investigated by a variety of methods, including high density DNA micro arrays.

Identification of molecular regulators involved in B cell apoptosis

Barbara Tiedt, Kurt Bommert in cooperation with Brigitte Wittmann-Liebold (MDC), Manfred Gossen (MDC)

In order to identify the regulators involved in B cell apoptosis, we have compared apoptotic and non apoptotic cells of the BL-60 cell line using two-dimensional gel electrophoresis and DNA micro arrays. We have identified an early response gene that is strongly upregulated shortly after anti-IgM addition. The high degree of homology with members of the CCCH family of tandem zinc finger proteins suggests that they promote the turnover of mRNAs containing class II AREs. We are currently investigating the link between mRNA turnover and apoptosis.

ORC (origin recognition complex) and MCM (minichromosome maintenance) proteins are essential for the replication of chromosomes. At least some of the genes mentioned above are turned off in quiescent or differentiated cells. Therefore, new synthesis is a prerequisite for proliferation. We have analyzed the expression of MCM and ORC proteins during apoptosis. After anti-IgM treatment, 70% of the BL-60 cells are apoptotic. Using H-3 thymidine assays, we have been able to show that the cells terminate proliferation. Western blot analysis revealed cleavage of MCM-3 protein into 80 kDa and 55 kDa fragments. Preincubation of the cells with caspase-3 inhibitor blocks MCM-3 cleavage as well as anti-IgM-induced apoptosis. In addition, we are analyzing the expression pattern of MCM and ORC mRNAs in samples derived from different disease stages of B-CLL patients.

Signaling and survival pathways in multiple myeloma

Dirk Hönemann, Manik Chatterjee, Suzanne Lentzsch, Tanja Lehmann, Ralf Bargou in cooperation with Axel Greiner (Würzburg) and Maingen GmbH (Frankfurt)

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 which is important for the survival and growth of myeloma cells is IL-6. We have been able to show that the IL-6-gp130-STAT-3 pathway is not essential for the survival of human myeloma cells, but might play a crucial role in the development of drug resistance. In addition, we have experimental evidence that the MAPK- and PI3K-pathways may be essential for the survival of malignant plasma cells. Therefore, we are currently investigating the activation mechanisms of these pathways to identify target structures for pharmacologic intervention. Based on these studies we plan to develop novel molecular therapy strategies.

Cytotoxic T-cell targeting by bispecific antibodies

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

We have shown that a novel recombinant bispecific single chain antibody, bscCD19xCD3, induces rapid and highly lymphoma specific 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 bscCD19-xCD3, bispecific antibody is able to induce nearly complete depletion of primary lymphoma cells mediated by autologous T-cells of patients with chronic lymphatic leukemia in the majority of cases analyzed. We plan to start a phase-I clinical trial of the treatment of patients with refractory B-cell lymphomas at the end of 2001. Furthermore, we are trying to establish a similar strategy for the treatment of multiple myeloma patients using a novel plasma cell-specific surface antigen as the target structure.

Apoptotic pathways in childhood T-ALL in the context of the maturation stage and clinical outcome

Leonid Karawajew, Christian Wuchter, Richard Ratei, Wolf-Dieter Ludwig

Within childhood T-ALL, cortical (CD1a positive) T-ALL has been identified as a subgroup having a favorable clinical outcome. We have investigated whether the different in vivo therapy response could be linked to a differential in vitro susceptibility to apoptotic cell death, and examined a large series of T-ALL leukemic cells (n=100) with respect to apoptosis-related features (expression levels of Bax, Bcl-2, CD95; susceptibility to spontaneous, CD95- and drug-induced apoptosis) as well as to their specific cytokine responsiveness in terms of apoptosis inhibition in vitro. Of the parameters investigated, only IL-7 rescue and dexamethasone-induced apoptosis exhibit a positive correlation with the cortical immunophenotype and a better early cytoreduction in vivo. IL-7 was also highly effective in inhibiting dexamethasone-induced apoptosis in vitro. Therefore, apoptosis-modulating pathways triggered by IL-7 and glucocorticoids are closely associated in T-ALL cells and may share common checkpoints responsible for the better in vivo-treatment response of cortical T-ALL.

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Molecular Immunotherapy

Antonio Pezzutto

Our group is working on the development of immunotherapy strategies for the treatment of renal cell carcinoma, colorectal carcinoma and chronic myeloid leukemia. In our GMP laboratory in the Robert Rössle Klinik we have completed the preparation of a cellular vaccine consisting of a gene-modified tumor cell line that expresses a shared renal carcinoma antigen recognized by T cells in context of HLA-A*0201 (developed in cooperation with Th. Blankenstein (MDC) and D. Schendel (GSF, Munich). We have also established GMP-certified production protocols for the generation of cellular vaccines consisting of peptide-loaded, tumor-lysate pulsed, or gene-modified dendritic cells.

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

Oliver Schmetzer, Christian Koch

Some patients with colorectal cancer develop an immune response to 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 has been established. Transgenic mice expressing human EpCam have been generated for use in preclinical vaccination experiments. Dendritic cells (DC) pulsed with recombinant EpCam protein or selected MHC-I and MHC-II peptides are being evaluated for their ability to induce rejection of EpCam-positive tumors. Multimeres of MHC-II binding EpCam epitopes are being prepared and evaluated in cooperation with K. Falk and O. Rötzschke (MDC). GMP procedures for the production of large quantities of recombinant EpCam protein are being optimized. The feasibility of a clinical vaccination study in patients with EpCam-positive adenocarcinomas is also being evaluated.

Use of dendritic cells for the induction of antileukemic immune response

Monika Schwarz, Beate Wittmann

Peptides derived from the bcr-abl fusion protein that is produced as a consequence of the t(9:22) chromosomal translocation in patients with chronic myeloid leukemia (CML) can bind to HLA-A3, -A11, or -B8. We have started a clinical trial using in vitro-generated, bcr-abl positive DC in CML-patients with the aim of inducing a CML-directed immune response. Therapy is safe and feasible and evaluation of immune responses is ongoing. In cooperation with B. Wittmann-Liebold, E. Müller and A. Otto, (MDC) we are analyzing naturally processed peptides from CML-cells in order to detect other potential candidate peptides for vaccination. We have established a bcr-abl specific ELISPOT assay for detection of bcr-abl specific T cells, an assay based on the use of HLA-Class I tetrameres is being developed. We have already detected bcr-abl specific T cells in some patients in clinical remission following interferon treatment. These assays will allow us to monitor anti-leukemic immunity in CML patients. Using 2-D gel electrophoresis (cooperation with A. Otto and E. Müller), we are evaluating CML cells treated with the tyrosine-kinase inhibitor STI571, in order to identify proteins that are expressed selectively in CML cells and that could be potential target for an immunotherapy approach.

Gene modification of dendritic cells

Jörg Westermann, Tam Nguyeng-Hoay

Both human and murine DC can be gene-modified using retroviral vectors and receptor mediated endocytosis (targeting the mannose receptor). Complexes of DNA, polyethiylenimine (PEI) and mannose are efficiently internalized, resulting in gene expression. A particular advantage of this method is the possibility of transferring several genes with the same construct, allowing expression of both tumor-antigens and genes that can modulate DC function, such as superantigens, chemokine receptors, and adhesion molecules. A stronger stimulation of the immune response or a change in the immune response with predominance of TH1 immunity can be achieved with this method. These studies are being performed in cooperation with M. Zenke (MDC). The use of cytokines that can modify the number and function of DC, such as Flt-3 Ligand and GM-CSF, is being investigated in gene transfer models in tumor vaccination experiments.

Role of ICOS Ligand in the regulation of the immune response

Günther Richter, Antonio Pezzutto,

The inducible co-stimulator receptor (ICOS) is a third member of the CD28 receptor family that regulates T cell activation and function. We have used soluble ICOSIg to further characterize the ICOS ligand (ICOSL) on APCs. We have also generated a new monoclonal antibody to ICOSL. ICOSL is expressed on monocytes, dendritic cells, and B cells. On CD34+ hematopoietic precursor cells, ICOSL is induced prior to CD80/CD86 induction suggesting that ICOSL is an early differentiation marker along the monocytic/dendritic matura-

tion pathway. Induction of ICOSL is dependent on TNF-α and is regulated via NF-κB. TNF-α-induced ICOSL expression seems to be functionally important for the co-stimulatory capacity of early hematopoietic precursors. The co-stimulatory capacity of leukemic cells at different maturation stages is being analyzed and correlated with ICOSL expression.

Selected Publications

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Molecular Immunology and Gene Therapy

Thomas Blankenstein

The group addresses questions related to immunology, tumor immunology and gene therapy. Examples of the group's activities during the report period are given below.

Interleukin-7/B7.1-encoding adenoviruses induce rejection of transplanted but not nontransplanted tumors

Most cancer vaccine trials are based on efficacy studies against transplanted mouse tumors that poorly reflect the clinical situation. We constructed adenoviruses expressing interleukin-7 and B7.1 and tested their therapeutic efficacy after transfer into established transplanted and non-transplanted 3-methylcholanthrene-induced tumors. The adenoviruses efficiently induced rejection of transplanted tumors, leaving behind systemic immunity. Against nontransplanted tumors of similar size, there were almost no therapeutic effects. This result was not due to the site of tumor development, tumor type, general immune suppression, or differences in transduction efficacy. Adenoviral expression of β-galactosidase as a surrogate antigen in nontransplanted tumors induced cytotoxic T cells that were unable to quantitatively reach the tumor site. Based on rigorous mouse models and an effective in situ immunization procedure, it is suggested that cancer vaccines can be effective, if at all, against "minimal residual disease"; additional experimental procedures must be found against established nontransplanted tumors.

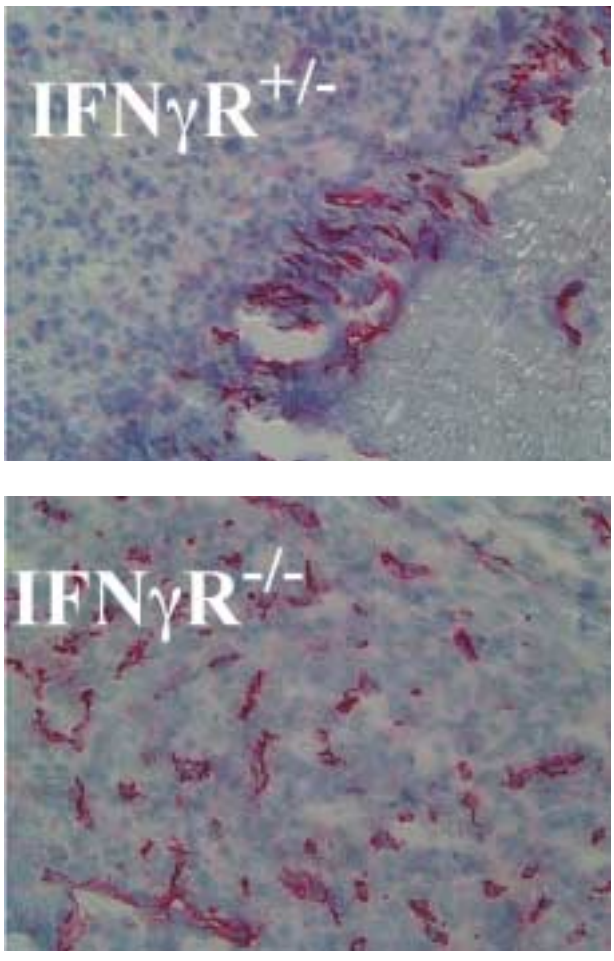
CD4+ T cell-mediated tumor rejection involves inhibition of angiogenesis that is dependent on IFNg receptor expression by nonhematopietic cells

Immunity against MHC class II$^-$ tumors can be mediated by CD4$^+$ T cells in the effector phase through an unknown mechanism. We show that this is IFNγ dependent but does not require IFNγ receptor (IFNγR) expression on tumor cells, T cells, or other hematopoietic cells and that IFNγR expression is not necessary in the priming phase. However, tumor immunity requires IFNγR expression on nonhematopoietic cells in the effector phase and involves inhibition of tumor-induced angiogenesis. This shows that an effective anti-tumor response involves communication between CD4$^+$ T cells and nonhematopoietic cells, most likely within the tumor stroma, and that tumor immunity must not entirely rely on direct tumor cell killing.

Decreased generation of anti-tumor immunity after intrasplenic immunization

The localization of antigen and the nature of the host antigen-presenting cells (APC) that present it to T cells are two major determinants of antigen immunogenicity. We analyzed whether the splenic microenvironment would support T cell priming and tumor immunity. We performed immunization/challenge experiments using different tumor cells known to induce CD8$^+$ cytotoxic T cells to a variable extent either exclusively by cross-priming (B7$^-$) or at least partially by direct priming (B7$^+$ tumors). Our results demonstrate that tumor take in the spleen required much less cells than at a subcutaneous injection site. Additionally, intrasplenic immunization was invariably ineffective compared to subcutaneous immu-

Tumor immunity requires IFNγ-receptor expression on non-hematopoietic cells and involves inhibition of angiogenesis. Shown is an immunohistological staining for blood vessels (α CD31 mAb). In immunized IFNγR-competent mice, blood vessels cannot grow into the tumor tissue. This tumor is rejected (top). In immunized IFNγR-deficient mice, blood vessels grow into the tumor tissue. This tumor grows progressively (bottom).



nization. We further showed that B cells were not responsible for the inefficient intrasplenic immunization. Therefore, delivering the tumor cell antigens inside the spleen by intrasplenic immunization did not improve but rather decreased the efficacy of tumor cell vaccines.

Efficient gene transfer into primary human CD8$^+$ T lymphocytes by MuLV-10A1 retrovirus pseudotype

Efficient and stable gene transfer into primary human T lymphocytes would greatly improve their use for adoptive transfer to treat acquired disorders, viral diseases, and cancer. We have constructed retroviral vector pseudotypes of amphotropic murine leukemia viruses (A-MuLV, MuLV-10A1), gibbon ape leukemia virus (GaLV), and feline endogenous virus (RD114) containing the enhanced green fluorescent protein (GFP) as a marker gene. Transduction of primary human CD8$^+$ T lymphocytes by the different GFP-retrovirus pseudotypes revealed the superiority of MuLV-10A1 in comparison with A-MuLV, GaLV, and RD114, respectively. The superior transduction efficacy of CD8$^+$ T cells by MuLV-10A1 correlates with its higher stability and, probably, the utilization of both the A-MuLV receptor (Pit2) and the GaLV receptor (Pit1) for cell entry.

Adoptive tumor therapy with T lymphocytes enriched through an IFNγ capture assay

Successful adoptive T cell therapy has been demonstrated in viral disease and selected forms of cancer. However, it is limited by the difficulty to efficiently isolate and amplify autologous tumor-reactive T cell clones. MHC class I-tetramers and peptide have greatly facilitated the characterization of CD8$^+$ T cells specific for tumor-associated antigens; yet for adoptive T cell therapy, MHC-tetramers have limitations: i) they require knowledge of tumor antigens which is often not available; ii) they select T cells with a single specificity, thereby, posing risk for selection of tumor escape variants; iii) they do not select for function, so that T cells may be anergic when isolated from cancer patients; and iv) they do not allow the isolation of CD4$^+$ T cells that can be essential for tumor rejection. Because IFNγ is essential for tumor rejection, we used a method to isolate live T cells based on their IFNg production. IFNγ secreted by previously activated T cells is retained on the cell surface, allowing their specific isolation and expansion. We showed that IFNγ$^+$ but not IFNγ$^-$ T cells from tumor-immunized mice are cytolytic and mediate tumor rejection upon adoptive transfer. Importantly, tumor-specific T cells can be enriched from lymphocytes infiltrating human renal cell carcinoma by the IFNγ capture assay.

Selected publications

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Cellular Immunology of Autoimmune Reactions

Kirsten Falk/Olaf Rötzschke

The driving force in the progression of autoimmune diseases is the autoreactive T cell. In most cases these autoreactive T cells are CD4+ T cells which have escaped the control mechanisms of the immune system. The group is currently interested in three basic problems related to the activation 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 but, up to now, these environmental factors have remained largely unknown. Recent experiments in our group, however, had demonstrated that small molecules carrying hydrogen donor groups (e.g. –OH) are able to trigger the exchange of peptide antigens on the surface of activated antigen-presenting cells. These antigens also include autoantigens (peptides and proteins), the target structure of autoreactive T cells. Studies by other groups have already shown that peptides derived from these autoantigens can induce fatal autoimmune reactions when loaded onto activated dendritic cells. Therefore, by catalysing this process, small molecular H-bond donor molecules might represent environmental risk factors which have not been considered yet. The group is currently defining the structural requirements of the compounds and investigating their impact in several experimental autoimmune model systems.

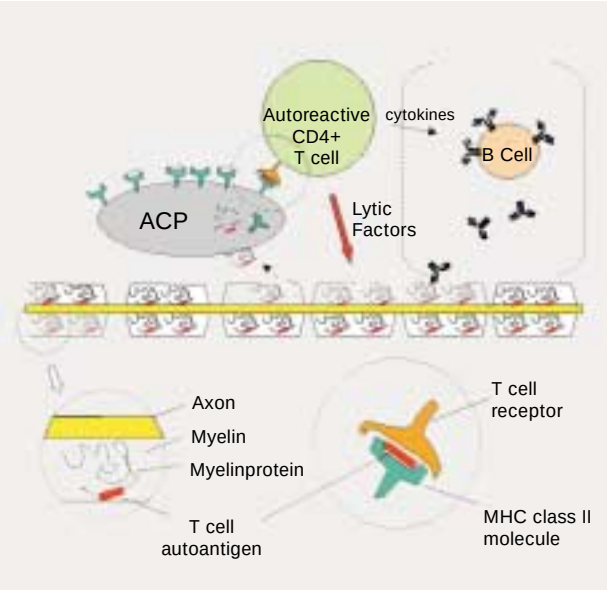
2. Control of autoimmune reactions

Several mechanisms had 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 appear to be 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 as one of the subpopulations responsible for this effect. The experimental tools in our group are T cell epitope oligomers (repetitive T cell antigens) and the heat shock protein gp96. Both have been previously found to be effective tolerance inducers *in vivo* and the use of these allows us to investigate the molecular basis of tolerance induction at the level of the T cell (oligomers) as well as at the level of the antigen presenting cell (gp96). The primary goals of these studies are the exploration of ways allowing a specific recruitment of these cells for the treatment of autoimmune diseases and the identification of key genes responsible for differentiation and maintenance of the suppressor status of regulatory T cells.

3. Selective activation of autoreactive 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 cells 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 have already been carried out in which the capacity of autoreactive CD4+ T cells has been tested with blood samples from tumour patients and in experimental mouse model systems. The T cell epitopes selected to trigger the autoimmune response represent tumour-associated antigens such as EPCAM (epithelial cell adhesion molecule) or self-antigens transfected into tumour cells as surrogate markers. The trials employ antigens with enhanced immunogenicity, such as epi-

Schematic mechanism of CD4+ T cell mediated tissue distruction (Multiple Sklerosis)



tope oligomers or lipo-peptides, and include the construction of inducible animal model systems (TET system). The goal of these studies is to investigate whether autoimmune responses driven by CD4+ T cells can in fact be used for tumour rejection.

Selected Publications

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Molecular and Cell Biology of Hematopoietic Cells

Martin Zenke

The research focus of this group is the molecular and cell biology of hematopoietic cells using human cells and, in experimental model systems, hematopoietic cells from mouse and chicken. Two hematopoietic cell types are being studied in detail: red blood cells and antigen presenting dendritic cells. Stem cells and early hematopoietic progenitor cells and their comittment and differentiation are also being investigated.

Determining the gene expression repertoire of red blood cells

Hacker, C., Lemke, B., Koh, K.-R., Anzinger, B., Blendinger, G , Knespel, S., and Bartunek, P.

Red blood cells represent one of the most abundant specialized cell types in vertebrate organisms. Their development from hematopoietic stem/progenitor cells is tightly controlled to ensure that they are produced in sufficient numbers to serve the needs of the organism both in the physiological and pathological states. We have now determined the gene expression repertoire of human red blood cells by DNA chip technology (in collaboration with Pfizer Inc., Groton, CT, USA) by employing an in vitro differentiation system of SCF/Epo-dependent erythroid progenitor cells (Panzenböck et al., Blood 92, 3658, 1998). The expression profile of more than 6000 annotated genes during erythroid cell differentiation has been determined. This analysis identified several molecules with a determining function in erythroid cell development that are currently being further analysed.

Thyroid hormone regulates the obesity gene, tubby

Koritschoner, N. P., Kurz, S. M., Heikenwälder, M. F., Bartunek, P., Blendinger, G.

The c-erbA protooncogene encodes a high affinity receptor for thyroid hormone (T3/T4; thyroid hormone receptor, TR). Our previous work established that TR/c-erbA has a decisive role in red blood cell differentiation and acts as a binary switch in determining erythroid cell fate: unliganded TR/c-erbA supports the growth of erythroid progenitor cells while ligand-activated TR/c-erbA induces their differentiation (Zenke et al., Cell 61, 1035, 1990; Bartunek and Zenke, Mol. Endo. 12, 1269, 1998). TR/c-erbA exerts its activity by activating and/or repressing gene expression, and several TR/c-erbA target genes have been identified. We have now focused on the analysis of the TR/c-erbA target gene, tubby. Tubby has been implicated in intracellular signalling downstream of insulin and G-protein coupled receptors and, when mutated in the tubby mouse, causes obesity and sensory deficiencies. We demonstrated that thyroid hormone regulates tubby in vivo and in vitro and, thus, revealed a molecular link between thyroid status and obesity (Koritschoner et al., 2001; in collaboration with A. Munoz, CSIC, Madrid, Spain; see also Heikenwälder et al., 2001). This is particularly interesting given the fact that thyroid hormone increases energy expenditure and thyroid hormone deficiency predisposes to weight gain and eventually obesity in humans.

Gene expression in antigen presenting dendritic cells

Ju, X.-S., Hieronymus, T., Hacker, C., Madruga, J., Kurz, S. M., Jorgas, T., Knespel, S., Euler, U.

Dendritic cells (DC) are professional antigen presenting cells that are unique in that they can initiate primary immune responses. However, many functional and molecular properties of DC remain poorly understood. We previously described an in vitro differentiation system for DC based on the conditional, hormone inducible v-rel estrogen receptor fusion gene v-relER (Boehmelt et al., Cell 80, 341, 1995). In this system DC differentiation and ontogeny were investigated, and v-relER DC were found to express lymphoid marker genes and, thus, appear to be related to the lymphoid subset of DC (Madruga et al., Immunobiology 202, 394, 2000).

To gain further insight into the underlying mechanisms that determine DC development, in vitro systems for differentiation of human and mouse DC from hematopoietic stem/progenitor cells have been developed. Cells are grown with a stem cell factor cytokine cocktail that maintains the progenitor phenotype and are induced to undergo synchronous differentiation into DC by administration of GM-CSF and IL-4. Differentiated cells express all the hallmarks of DC as judged by morphology, surface marker expression, functional activities and their gene expression profile, and can be induced to mature by TNFα. The genetic program that determines DC development from hematopoietic stem/progenitor cells is being determined by employing DNA chip technology (in collaboration with J. Reich et al. and C. Scheidereit et al., MDC, Berlin). Several genes, that have not been implicated in DC development and function, and novel genes have been identified and are being studied using knockout mice strains.

For example, DC express c-met/scatter factor (SF) receptor and activation of c-met/SF receptor by cognate ligand was demonstrated to induce exit of Langerhans cells (LC), the cutaneous contingent of DC from skin (Kurz et al., Eur. J. Immunol., in press; in collaboration with W. Birchmeier et al., MDC, Berlin).

Gene transfer into antigen presenting dendritic cells (DC)

Gust, T. C., Hieronymus, T., Gontscharenko, M. and Diebold, S. S.

Given their unique properties in antigen-specific T cell activation, DC represent a particularly attractive cell type for use in the immunotherapy of disease. In peripheral organs (for example in skin) DC are exposed to a variety of pathogens, such as viruses and bacteria, which they capture through specific cell surface receptors. Accordingly, gene-modified DC were generated that capitalize on using such surface receptors for gene delivery into DC by receptor-mediated endocytosis (in collaboration with M. Cotton, IMP, Vienna, Austria; E. Wagner, Boehringer Ingelheim Austria R&D, Vienna, Austria; J. Westermann and A. Pezzutto, Charite, Robert-Rössle-Klinik, Berlin).

Mannose polyethylenimine (ManPEI) and adenovirus polyethylenimine (Ad/PEI) DNA transfer complexes were effective in delivering DNA and RNA into human and mouse DC, and eliciting specific MHC class I restricted T cell responses (Diebold et al., Hum. Gene Ther. 10,775, 1999; Diebold et al., J. Biol. Chem. 274, 19087, 1999; T. Gust and M. Zenke, unpublished). We have now also generated chimeric transferrin receptor (TfR) and invariant chain (Ii) fusion genes with ovalbumin (OVA) antigen to target the MHC class II processing pathway. Such TfR-OVA and Ii-OVA fusion genes were found to effectively produce both CD8 cytotoxic T cell responses and CD4 T cell help (Diebold et al., 2001; in collaboration with N. Koch, University of Bonn, Bonn, Germany). Thus, modification of the antigen encoding cDNA represents a convenient way to direct endogenously expressed antigens to MHC class II presentation and, thus, generate T cell help.

Stem cells and cell fate in hematopoiesis

Bartunek, P., Kirsch, R. D., Hacker, C. , Hieronymus, T. and Blendinger, G.

All mature cell types of the hematopoietic system develop from hematopoietic stem cells through successive steps of commitment and differentiation. To study the underlying mechanisms that determine cell fate and lineage choice, hematopoietic stem/progenitor cells were isolated from 2 day old chicken embryos and mouse bone marrow (in collaboration with M. Dvorak, IMG, Prague, Czech Republic and A. Müller, University of Würzburg, Würzburg, Germany). In the chicken system, early bFGF-dependent progenitors were isolated with a differentiation potential confined to both the erythroid and myeloid lineage (Karafiat et al., 2001; Bartunek et al., 2002). Also, in the mouse system, we are currently screening for and analysing genes that are important for the establishment and maintenance of the stem cell phenotype and for lineage choice (Hacker et al., manuscript in preparation; in collaboration with G. Kempermann, MDC, Berlin; A. M. Wobus, IPK, Gatersleben, Germany and W. Huttner, MPI, Dresden, Germany).

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Thyroid hormone regulates the obesity gene tub. EMBO Reports 2, 499-504.

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Cell Cycle Regulation and Gene Therapy

Research Group of the Humboldt University Berlin at the MDC

The role of the tumor suppressor DPC4 in TGF-β signaling and its implications for cancer progression

Susanne Boeckh

TGF-βs act as potent growth suppressors in the majority of mammalian cells, whereas many cancers are resistant to TGF-β. Especially in tumors derived from pancreatic and colon epithelial cells, impairment of the TGF-β pathway, as manifested by genetic alterations of TGF-βs, their receptors, or elements of the associated signaling cascade such as the Smad proteins, have been observed. In order to understand the acquisition of a malignant phenotype in pancreatic carcinoma we are aiming to identify alterations in gene expression induced in pancreatic and colon cancer cells by TGF-β. For this purpose we use cDNA microarray technologies to compare the transcript pool of cells harboring a mutant Smad4 protein (also called DPC4 for ***d***eleted in ***p***ancreatic ***c***arcinoma) with their reconstituted counterparts. Moreover, our goal is to analyse the temporal program of transcription in response to TGF-β by comparing expression profiles at different timepoints of induction. Using this approach we intend to draw a comprehensive picture of the signaling network of the major TGF-β responsive genes and, in particular, try to understand the role of DPC4 in the TGF-signaling cascade. We hope to identify target genes suitable for a pharmacological or gene therapeutic approach to cancer therapy. We are currently focussing on a prominent cluster of serine protease inhibitors (serpins) involved in suppression of invasion and inhibition of angiogenesis whose expression is solely dependent on the presence of DPC4 and, moreover, localized on the same chromosome downstream of this tumor suppressor. The activation of this cluster is likely to be one of the major mechanisms by which DPC4 prevents tumor growth and metastasis.

Gene therapy of malignant tumors

Karsten Brand, Sefer Eleskurtaj, Martina Geheeb, Christina Montag, Ansiah Shakeri-Garakani, Michael Sachariat, Marianne Wedde

This group deals mainly with the development of new methods of virus-based gene therapy of tumors, especially colorectal liver metastases. We have basically adopted three approaches:

1. Gene therapeutic chemotherapy by intratumoral gene transfer of the Herpes Simplex Virus thymidine kinase gene which renders intravenously applied Ganciclovir toxic.
In previous studies, we have described the toxicitiy of this approach and the possible underlying mechanisms. We then demonstrated how to overcome this toxicity with a marked degree of anti-tumor efficacy by using the tumor tissue-specific CEA promoter instead of the ubiquitously expressed CMV promoter. Currently, we are constructing gutless adenoviral vectors which are less toxic than first-generation adenoviruses. We are also examining a variety of ameliorated tumor tissue-specific promoters in the context of this new vector generation aimed at clinically relevant therapy with high efficacy but low toxicity.

2. Transfer of cell cycle inhibitory and apoptosis-inducing genes.
We were able to show the therapeutic relevance of the simultaneous expression of more than one gene of these classes of proteins. We have examined the interactions of the apoptosis inducer p53 and the cell cycle modulators pRb and E2F. The nature of these interactions can in turn be exploited for gene therapeutic applications.

3. Inhibition of the invasion of micrometastases by the transfer of protease inhibitors into the unaffected tissue of host organs to confer a defensive function.
We have recently shown that the adenoviral gene transfer of inhibitors of tumor cell asssociated proteases leads to a dramatically decreased growth of metastatic deposits in the liver of mice after injection of highly metastatic cells into the spleens of these animals. We are currently trying to construct modern viral vectors (AAV, gutless Ads) with protease inhibitor genes aimed at combining this very efficient approach with the lowest possible toxicity.

Gene therapy of monogenetic liver diseases

G. Cichon in collaboration with T. Benhidjeb and P.M. Schlag

The goal of this project is the development of viral gene transfer systems suitable for long-term expression of therapeutic genes in the liver of mammals. This project includes three different topics:

In the frame of the first topic, possible adverse side-effects of viral gene therapy (especially adenoviral gene therapy) are explored. The effect of systemic high dose applications on cellular and humoral components of the blood and inflammatory changes of the organs (in rabbits, rats and mice) are being analysed and prophylactic measures are being explored regarding their protective potential.

A second topic is the development of viral hybrid vectors which combine the excellent in vivo gene transfer efficiency of recombinant adenoviruses and the single property of lentiviral vectors to integrate their genes into chromosomes of resting cells. During this part of the project, new techniques for large scale production of lentiviral vectors are also being explored.

The third topic of the project is the development of an animal model suitable for long-term expression studies of therapeutic genes. Long-term gene expression of therapeutic genes is often hindered by a combination of factors including immunological defense mechanisms of the host and physiological overload of transgenic cells. To differentiate between the impact of immunological reactions and cellular changes which could be attributed to the permanent expression of the transgene two mice strains were bred which combined a monogenetic defect (low density receptor deficiency; LDL-R k.o. mice) and a severe combined immunodeficiency (SCID mice) in one animal. In this model long-term expression studies after transfer of an LDL-receptor gene are being performed.

Selected publications:

Cichon, G., Schmidt, H., Benhidjeb. T., Löser, P., Haas, R., Grewe, N., Ziemer, S., Schnieders, F., Heeren, J., Manns, M., Schlag, P., and Strauss, M. (1999) Intravenous application of recombinant adenoviruses causes thrombocytopenia, anemia and erythroblastosis in rabbits. J Gene Med. 1: 360-371

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Evolution, Regulation and Genetic Applications of Transposable Elements in Vertebrates

Zoltán Ivics

Work in the "Transposition" group involves transposable DNA elements. In the past years we have laid the foundations for the use of *Sleeping Beauty* (SB), a synthetic transposable element, as a molecular tool for vertebrate genetics. We are now concentrating our efforts on the following research areas.

1. SB has a number of advantages as a gene vector when compared with 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 a useful 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. We are evaluating various viral and non-viral gene delivery agents in combination with transposon vectors to derive high-efficiency gene transfer and long-term gene expression *in vivo*.

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 a transposon-based insertional mutagenesis screen in the zebrafish, using gene-trap transposon vectors in which the expression is dependent on transposition into transcribed genes. Spatial and temporal patterns of reporter expression can be colocalized 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. **Transposons have learned how to coexist peacefully for millions of years with their host cells. We are investigating how transposition is regulated in vertebrates, and the molecular interactions through which that peaceful relationship has been achieved during evolution.**
   a) We are following a "candidate" approach, by looking for interactions with cellular factors that are involved in other recombination systems. We have established that high mobility group proteins as well as proteins that are involved in double-strand DNA break repair are host factors for transposition. We are in the process of investigating the involvement of other repair and/or cell cycle checkpoint proteins in transposition.
   b) We are also following a "blind" approach, by performing a yeast two-hybrid screen of a human gene library. With this screen, we have already identified two human proteins that specifically interact with the Sleeping Beauty transposase.

   In our search for gene regulatory networks that are activated in response to transposition, we are in the process of identifying relevant transcriptional changes in gene expression by using Affymetrix gene chips. This approach allows us to gain insight into the complex regulation of transposition in vertebrate cells.

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Experimental Pharmacology

Iduna Fichtner

In recent years the research focus of the group has concentrated mainly on two topics:

- Growth regulation and therapeutic approaches to breast carcinomas
- Differentiation and engraftment of stem cells

The main tool for our investigations is a very broad panel of human tumours used as in vitro or in vivo experimental models. We work closely with clinicians in order to achieve clinically relevant results.

Breast carcinoma research

Based on long experience in this field and on the availability of several xenotransplantable tumour lines resembling the clinical characteristics of the tumour entity (hormone receptor status, response to cytostatic or endocrinological therapy), we are interested in the phenomenon of antiestrogen resistance. About one half of breast cancer patients are treated in first-line therapy with the antiestrogen tamoxifen. Unfortunately, a substantial number of breast carcinomas fail to respond initially and, after several months of therapy, almost all tumours become resistant. We mimicked the clinical situation by treating nude mice bearing a breast carcinoma xenograft (3366) with tamoxifen. After two years we succeeded in developing a tamoxifen-resistant subline (3366/TAM) which maintained its oestrogen receptor (ER) positivity. There were no mutations found in the ligand-binding domain of the ER. Surprisingly, regulation mechanisms for the ER and ER-related genes (progesterone receptor, cathepsin D, pS2) had changed due to the acquired resistance phenotype.

Additionally, different immuno-reactivities of ER towards a specific antibody in the Abbott enzyme immunoassay were found. The invention was patented and the observation was verified in preclinical studies and a first retrospective clinical study.

These results led us to the hypothesis that co-factors - outside the direct hormone signalling pathway - are involved in the antiestrogen resistance phenotype. Studies to elucidate different gene (microarray technique) and protein (2D-electrophoresis) expression patterns in antiestrogen-sensitive and -resistant breast carcinomas were initiated with partners inside or outside the MDC.

One approach to overcome tamoxifen resistance has led to the invention of a special liposomal formulation involving vesicles which were therapeutically active in in vitro (cell culture) and in vivo (xenografts) assays and even after oral administration. This invention was patented and will be developed for clinical use. The mechanisms used by this new drug formulation to overcome resistance are still unclear. We believe differences in pharmacokinetics, cellular uptake (p-glycoprotein binding), intracellular trafficking (antiestrogen binding sites) may be involved and have started investigations in these areas.

Stem cell research

Acute leukemias represent a phenotypically and morphologically heterogeneous group of haematological diseases developing from preleukaemic states and known to be incurable in most cases.
In acute leukaemia, phenotypic and functional alterations are observed in bone marrow stromal cells indicating disturbed function of the stromal compartment which might promote the proliferation of leukaemic cells.

Following transplantation of CD34-positive human blood progenitor cells into immunodeficient NOD/SCID mice, we observed the engraftment and proliferation of human stromal progenitors along with human haematopoietic cells. This xenotransplant model was then used to investigate the role of circulating stromal progenitors obtained from patients with acute leukaemia and from normal donors for the engraftment and proliferation of leukaemic versus normal haematopoietic stem cells. Alterations with unknown ethno-pathologic significance were observed in the stromal compartment of patients suffering from acute leukaemia, including a reduction in fibroblastic and adipocytic elements as well as induced proliferation of endothelial cells, leading to pronounced angiogenesis within the bone marrow.

The self-renewing and repopulating potential of primitive haematopoietic stem cells can only be demonstrated in vivo. At present, immunodeficient mice accepting xenotransplants like the NOD/SCID mouse are being used to investigate the engraftment of long-term-repopulating human stem cells. They have also been used successfully to visualise the existence of leukaemic stem cells in acute leukaemia suggesting that the leukaemic transformation might occur at the level of the early haematopoietic stem cell. Up to now, there have been no investigations concerning the presence of stromal progenitors within the leukaemic grafts and their significance for the establishment of normal versus leukaemic haematopoiesis. The figure shows results concerning the occurrence of stromal cells in bone marrow of mice transplanted with an human acute myeloid leukaemia (AML).

The main objective of our studies is to gain insight into the mechanism of leukaemic cell proliferation in vivo and the role of circulating stromal progenitors for the establishment of human acute leukaemia in NOD/SCID mice. Results from these studies are expected to be of clinical relevance in terms of prognostic evaluations and for the development of new therapeutic strategies.

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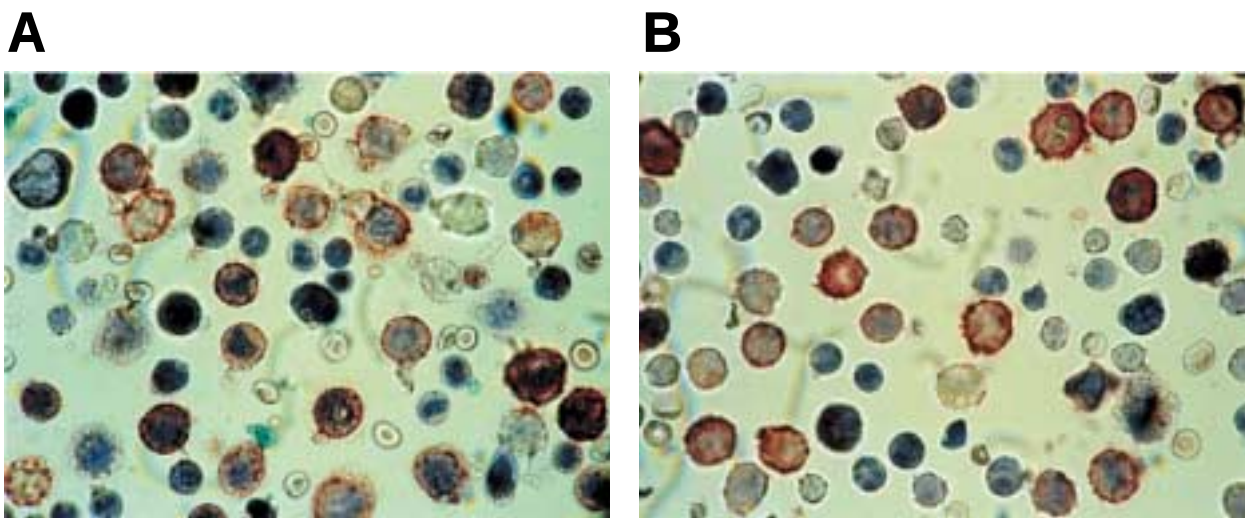
Detection of human leukaemic and stromal cells in the bone marrow of NOD/SCID mice six weeks after transplantation of AML-cells. Immunocytology with antibody against: A: CD13 (myelocytes); B: EN4 (endothelial cells).



Drug Targeting

Regina Reszka

The major focus of our group is the development, characterisation, and testing of new drug carrier systems based on liposomes for both therapeutic and diagnostic applications. Therapeutic approaches include the establishment and optimisation of in vivo liposomal gene transfer of "suicide genes" as well as p53-independent apoptosis-inducing genes all for use in the treatment of primary brain tumors and liver metastases.
For the development of rational therapeutic approaches the gene expression abnormalities in human glial tumors will be identified by gene array analysis.

Gene expression abnormalities in human glial tumors

To gain more genetic and basic molecular information about the growth characterisation and invasiveness of recurrent glioblastoma, we have developed and expanded a brain tumor bank in co-operation with the neurosurgery unit of Berlin-Buch. This resource involves an extensive catalogue of CNS tumors including different stages of glioblastoma and astrocytoma (grade II, III, IV). We have started work on the gene expression profiling using 15 glioblastomas. For the gene array analyses, poly(A)+ mRNA from normal and tumor tissues has been isolated, labeled with ^{33}P and hybridized to the membranes. The Gene Discovery Array Human Unigene Set II (RZPD) (including three 22x22 cm nylon filters) is spotted with more than 27.000 non-redundant human cDNA clones per filter. In the first run we found more than 200 EST sequences apparently expressed differentially.

Therapy of glioblastomas - Clinical phase I/II study

In a two arm clinical phase I/II the non-viral suicide gene delivery system DAC-30™/HSVtk **LIPO-HSV-1-tk** (DAC-30™: DAC-CHOL/DOPE); HSVtk: *Herpes simplex* virus thymidine kinase gene) has been evaluated involving the neurosurgery centers in Cologne and Düsseldorf.

In the first arm, patients with non-resectable glioblastomas receive a targeted high flow infusion of the **LIPO-HSV-1-tk** gene transfer system via two stereotactically placed catheters within the tumor. Ganciclovir treatment over 14 days was carried out starting 4 days after 3- day pump application of the **LIPO-HSV-1-tk** gene transfer system. Positron-emission tomography (PET) with I^{124} labelled 2'-fluoro-2'deoxy-1β-D-arabino-furanosyl-5-Iodo-uracil ([I^{124}] FIAU) – a specific marker substrate for gene expression of HSV-1-tk – was used to identify the location, magnitude, and extent of vector-mediated HSVtk gene expression for the first time in humans. The preliminary findings in a small patient group of 5 show that FIAU-PET-imaging of HSV-1-tk expression is feasible and vector-mediated gene expression may predict the therapeutic effect (Fig. Co-registration of FIAU-PET, MET-PET, FDG-PET and MRI before and after vector application).

In the second arm, patients with resectable tumors were included. After surgical removal of the main glioblastoma mass, a catheter system is placed in the former marginal zone of the tumor followed by the treatment schedule as described above.

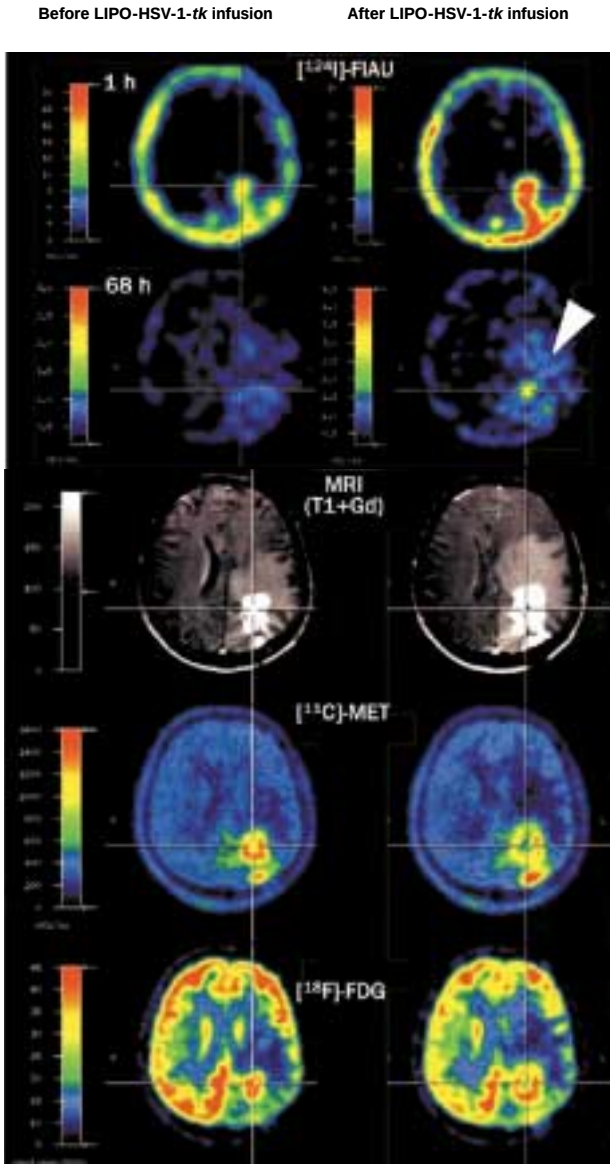
We have developed, in co-operation with Prof. Winters group in Dortmund and Dr. Pampel in Leipzig, an implantable drug depot formulation encapsulating clinically well established cytostatics with known dose-limiting toxicities like Carboplatin and Taxol. This novel system represents a cubic phase structure which releases both encapsulated drugs with a different sustained phamacokinetic behaviour. It will be used for the local chemotherapy of glioblastomas after surgery and will be adapted for gene transfer.

Therapy of liver metastases

In contrast to the viral-based strategies, our delivery approach uses *in vivo* cationic and surface-modified liposomal gene transfer systems applied intrahepatically. For the effective transfer of marker and therapeutic genes (including suicide genes), we have developed a new drug carrier embolisation system (DCES) which combines three novel principles to yield a hybrid technique with high transduction and therapeutic efficiencies.
Presently, our in vivo studies with rats are focused on optimisation of the HSVtk-DCES gene therapy tool. Different prodrugs (ganciclovir, acyclovir and valacylovir) are being compared to evaluate the efficacy and associated toxicity after i.v., i.p. or oral administration.

Characterization of the DNA interactions with different peptids, lipids and dentrimeres as well as cell or nuclear uptake mechanisms of non viral gene transfer systems

Another interest is the characterisation of two novel gene transfer systems from Qiagen, SuperFect™ and Effectene™. SuperFect™ is an activated dendrimer and Effectene™ a novel cationic lipid including an additional DNA-condensing agent. The special focus of these studies is characterisation of the electrostatic and colloidal properties which give rise to effective gene transfer.



GMP signalling via cGKIa is important for the correct pathfinding of sensory axons. The pathfinding of sensory axons within the developing spinal cord was analyzed. Nociceptive axons in (A) wild-type (+/+) and in (B) cGKI-deficient mice (-/-) of transverse sections of the spinal cord. In the absence of cGKI sensory axons leave the dorsal funiculus prematurely and grow towards the central canal (arrowheads). C, central canal; E14, embryonic day 14.

To obtain detailed knowledge about the molecular regulation of phospholipid assymetry and the influence of the calcium sensing receptor on the uptake mechanism of non-viral gene transfer systems into tumor cells we have studied calcium signaling processes in different cell lines.

Additionally, to enhance the nuclear transport of transduced DNA peptides harboring nuclear localizing sequences (NLS) were complexed with plasmid DNA. Our biophysical and in vitro studies indicate that a broad spectrum of factors influence the improvement of nuclear transport.

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Inhibitors of telomerase as potential anticancer agents

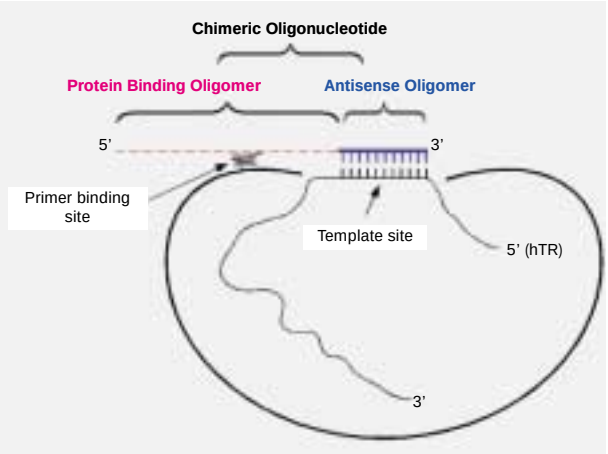
The end of mammalian chromosomes consists of telomeres. These are repeats of a short DNA sequence (TTAGGG; average length of 5-15 kb) and are associated with proteins. These complex structures protect chromosomes against end to end fusion and prevent telomere ends being recognized as DNA breaks, triggering repair, cell cycle arrest or apoptosis. However, as a consequence of the DNA synthesis mechanism, some of the telomeric repeats are lost during each round of cell division. When telomeres reach a critically short length, cells enter a state termed replicative senescence, which can induce apoptotic cell death. This is characteristic of most somatic cells.

In contrast, cancer cells, germ cells and some stem cells escape such replicative limitations by the expression of telomerase which is capable of synthesizing telomeric DNA. Inhibition of telomerase is, therefore, considered as a strategy for changing the immortal state of a cancer cell to a mortal one. Telomerase is a unique ribonucleoprotein polymerase consisting of an RNA (TER) which contains the template for synthesis of telomere DNA and a catalytic protein, the telomerase reverse transcriptase (TERT) which has homology with viral reverse transcriptases. Therefore, it was not surprising that some of the RT inhibitors of HIV emerged as inhibitors of telomerase.

Based on our experience in designing, synthesizing and evaluating reverse transcriptase inhibitors against human immunodeficiency virus and hepatitis B virus, we have investigated triphosphates of more than 50 nucleoside analogues, (including 11 newly synthesized) as possible inhibitors of telomerase. A leading structure was found which seems to fulfill the general requirements for an efficient suppressor of human telomerase. We are now trying to optimize its structure, especially to improve its intracellular stability and selectivity.

Oligonucleotides (ODNs) are a second group of compounds we have developed as inhibitors of telomerase. They are differently modified at the 5'- and the 3'-ends to address different targets of telomerase. The design of these so-called chimeric ODNs (cODNs) is based on our surprising finding that phosphorothioate modified ODNs (PS-ODNs) do not cover the RNA but bind strongly to the primer binding site of telomerase protein (TERT), displacing the primer from its binding site and causing a strong inhibition of telomerase. This inhibition by PS-ODNs is more length- than sequence-dependent. The extension at the 3′-end of PS-ODNs by a second oligomer, which is effective as antisense ODN against the RNA template, results in cODNs which are active in the subnanomolar range (Figure). Length variations of the PS-part (10-20mer) and structural modifications of the antisense part (2'-O-methyl, phosphoramidate, peptide nucleic acids (PNA)) of the cODNs have shown that PS-PNAs (synthesized by Dr. E. Uhlmann, Aventis) are highly effective inhibitors of telomerase at a cellular level, even 6 days after transfection with lipofectin (U-87 glioblastoma cells: ID_{50}= 0.05 µM). However, intravenous application of a series of PS-PNAs to nude mice carrying human U-87 tumors had no effect on the telomerase activity of the tumor cells (cooperation with Dr. I. Fichtner, MDC). This might be due to inadequate uptake of PS-PNAs under in vivo conditions, in spite of their surprising effects at the cellular level.

Ongoing research in our laboratory is directed towards selecting appropriate oligonucleotides for in vivo applications.



Model of the human telomerase. Two functionally essential parts, the primer binding site of its protein and the template site of its RNA can be inhibited simultaneously by a single but differently modified oligonucleotide (chimeric oligonucleotide).

New imaging agents for detection of human tumors by PET

Positron emission tomography (PET) may be an excellent procedure for the detection of human tumors as well as for the estimation of the tumor response to cancer therapy. However, its applicability is limited by the lack of imaging agents specific for tissue and tumor proliferation. [F-18] Fluorodeoxyglucose is nearly the only agent used for tumor imaging in

clinical practice but it is more an indicator of glucose metabolism than of tumor proliferation. Therefore there is an urgent need for specific tumor imaging agents.

We have designed, synthesized and investigated a series of modified thymidine analogues to meet the following biological and chemical requirements we consider essential for successive tumor imaging with thymidine analogues.

1. They must be taken up by tumor cells and phosphorylated well by thymidine kinase, an enzyme which increased about 10-20 fold when cells synthesize DNA. The phosphorylated products are trapped and accumulate inside of proliferating cells.
2. The modified thymidine analogues must be resistant to pyrimidine nucleoside degrading enzymes, thereby, avoiding positron-emitting cleavage products distributing nonspecifically in the body.
3. The chemical labeling and purification procedure of tracer amounts of the products must be simple and rapid.

We have selected some compounds which seem to be highly attractive for our aims. One of these analogues is 5–monofluoro-methyl-3´-fluorodeoxyuridine, in which the imaging [F-18] fluorine group is introduced into the methyl group. The next step will be to adapt the [F-18] labeling procedure to an automated synthesis. These labeling- and all tomograph-dependent preclinical experiments in animal tumor models and a possible pilot study with lung cancer patients will be carried out in cooperation with the Clinic of Nuclear Medicine of Humboldt University (Charité), Berlin.

Selected Publications

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Molecular and Developmental Neurosciences

Molecular and Developmental Neurosciences

As the average life-expectancy continues to increase in the Western hemisphere, it is anticipated that the incidence of age-related disorders, such as Alzheimer's disease, will also rise. This expectation has stimulated renewed interest in the neurosciences. In the past few decades, significant advances have been made in our understanding of the functional basis of the nervous system. Nevertheless, despite the rapid growth in neuroscience research at the international level, considerable progress remains to be made in the elucidation of those molecular events that underly brain disorders.

Due to the immense complexity of the brain, progress in understanding the molecular processes of the brain's function has been slow. However, two different approaches have helped combat this problem. On one hand, Positron Emission Tomography (PET) and Nuclear Magnetic Resonance Imaging (NMR) enable neuroscientists to visualize and map active centers of the brain in relation to specific functions. On the other hand, molecular and cell biology tools are employed to study the properties and behavior of single brain cells. The combination of these two approaches should lead to an improved understanding of higher brain function, and will help in the design new treatments for the specific degenerative mechanisms which lie at the root of many brain diseases. The neuroscience program at the MDC is focused on a molecular approach. This perfectly complements the brain imaging activities at the Jülich Research Center since both centers constitute the neuroscience program of the Helmholtz Society.

The research groups of the MDC neuroscience program focus on both molecular and cellular approaches. Helmut Kettenmann´s group investigates the role of glial cells in health and disease, while the Developmental Neurobiology group, under the direction of Fritz G. Rathjen, analyzes molecular aspects of axonal growth and synaptic plasticity of the central nervous system. Gary R. Lewin and his coworkers are identifying novel genes responsible for mechano-transduction and their regulation by neurotrophins. Christiane Alexander is identifying the genes involved in neurodegeneration. Gerd Kempermann´s group studies stem cells in the adult nervous

Molekulare Neurowissenschaft und Entwicklungsneurobiologie

Da die durchschnittliche Lebenserwartung in der westlichen Hemisphäre weiter zunimmt, ist auch damit zu rechnen, dass die Zahl an altersbedingten Krankheiten wie etwa Morbus Alzheimer weiter steigen wird. Diese Erwartung hat zu einem neuerlich wachsenden Interesse an den Neurowissenschaften geführt. In den vergangenen Jahrzehnten konnte das Verstehen der funktionalen Basis des Nervensystems deutlich verbessert werden. Nichtsdestoweniger werden unabhängig von dem raschen Wachstum in den Neurowissenschaften auf internationaler Ebene noch viele weitere Fortschritte benötigt, um die molekularen Ereignisse aufzuklären, die den Erkrankungen des Gehirns zugrunde liegen.

Wegen der ungeheuren Komplexität des Gehirns kommt der Fortschritt beim Verständnis der zu den Gehirnvorgängen gehörenden molekularen Vorgänge nur langsam voran. Zwei Ansätze konnten inzwischen genutzt werden, um gegen dieses Problem anzukommen. Da sind auf der einen Seite die Positronen Emissions Tomographie (PET) und die Kernmagnetresonanz-Bildgebung (NMR Imaging, NMRI), mit deren Hilfe die Neurowissenschaftler aktive Zentren des Gehirns in Relation zu spezifischen Funktionen sichtbar machen und aktiv kartieren. Auf der anderen Seite werden die Werkzeuge der Molekular- und der Zellbiologie benutzt, um die Eigenschaften und das Verhalten einzelner Nervenzellen zu analysieren. Die Kombination beider Ansätze sollte zum einen zu einem verbesserten Verständnis der höheren Hirnfunktionen führen, und sie wird zum zweiten helfen, neue Behandlungsmethoden für die spezifischen degenerativen Mechanismen zu entwickeln, die an der Wurzel vieler Hirnerkrankungen liegen. Die Forschungen am MDC komplettieren auf perfekte Weise die Bildgebungsaktivitäten am Forschungszentrum in Jülich, da beide Zentren das neurowissenschaftliche Programm der Helmholtz Gemeinschaft ausmachen.

Die Forschungsgruppen des MDC-Programms für die Neurowissenschaften konzentrieren sich sowohl auf den molekularen als auch auf den zellulären Ansatz. Die Gruppe von Helmut Kettenmann untersucht die Rolle der Gliazellen in gesundem und kranken Gewebe, während die Gruppe Ent-

system in order to understand the role of these cells in brain plasticity. Erich Wanker´s group will use high-throughput functional genomics approaches for the identification and characterization of novel drug targets involved in neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases. Furthermore, screening assays for the identification of small molecules preventing protein aggregation in late-onset neurodegenerative diseases will be developed.

The central theme of the MDC is to link basic and clinical research. We have, therefore, established collaborations with the Neurosurgery Department in Berlin-Buch and the Charité with a focus on brain tumors and with the Department of Neurology at the Charité with a focus on brain inflammation and neural stem cell research. These interactions are encouraged since we are part of the Collaborative Research Center (Sonderforschungsbereich) and the Graduiertenkolleg at the Charité established to study the role of brain cells in the pathogenesis of CNS diseases.

wicklungsneurobiologie unter der Leitung von Fritz G. Rathjen die molekularen Aspekte des axonalen Wachstums und der synaptischen Plastizität des Zentralen Nervensystems analysiert. Gary R. Lewin und seine Mitarbeiter identifizieren neuartige Gene, die für die mechanische Übertragung und ihre Regulierung durch Neurotrophine verantwortlich sind. Christiane Alexander befaßt sich mit der Identifizierung von Genen, die an der Neurodegeneration beteiligt sind. Gerd Kempermanns Gruppe studiert Stammzellen im erwachsenen Nervensystem, um die Rolle dieser Zellen in der Plastizität des Gehirns zu verstehen. Erich Wankers Gruppe wird die neuen Ansätze der funktionalen Genomik mit hohen Durchlaufraten einsetzen, um neuartige Ziele für solche Arzneimittel zu identifizieren und zu charakterisieren, die Einfluß auf neurodegenerative Erkrankungen wie Alzheimer, Parkinson oder Huntington haben. Darüber hinaus werden Verfahren für Reihenuntersuchungen entwickelt, mit denen kleine Moleküle identifiziert werden können, die die Proteinaggregation verhindern, die bei spät einsetzenden neurodegenerativen Erkrankungen beobachtet werden.

Das zentrale Thema des MDC ist die Verbindung von grundlegender und klinischer Forschung. Wir haben daher Kollaborationen zum einen mit der neurochirurgischen Abteilung in Berlin-Buch und der Charité begonnen, in deren Fokus Hirntumoren stehen, und wir haben zum zweiten eine Kollaboration mit der Abteilung für Neurobiologie an der Charité begonnen, in deren Fokus die Erforschung von Hirnentzündungen und neuronalen Stammzellen steht. Diese Wechselwirkungen werden durch die Tatsache ermutigt, dass wir zu einem Sonderforschungsbereich und dem Graduiertenkolleg gehören, die beide von der Charité eingerichtet worden sind, um die Rolle von Hirnzellen in der Pathogenese der Erkrankungen des Zentralen Nervensystems zu erkunden.

Neurodegeneration

Christiane Alexander

Autosomal dominant optic atrophy (ADOA) is the commonest hereditary optic neuropathy resulting in progressive loss of visual acuity, centrocoecal scotoma, and bilateral temporal atrophy of the optic nerve with an onset during the first two decades of life. ADOA occurs with an estimated prevalence of between 1:12,000 (Denmark) and 1:50,000. The disease is highly variable in its 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.

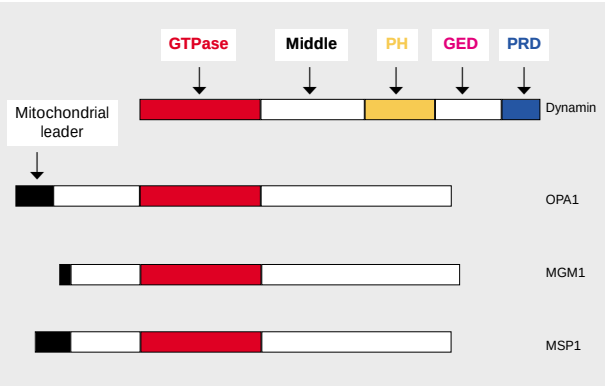
OPA1 – the gene responsible for autosomal dominant optic atrophy

The predominant locus of 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. We set out to identify the OPA1 gene using a positional cloning approach and constructed a physical map based on a high density PAC contig covering the entire OPA1 candidate region. For the identification of candidate genes, large-scale sequence sampling was performed on PACs representing the minimal tiling path for the OPA1 interval. EST SHGC37414 was found to map to PAC H20545, and the corresponding full-length cDNA, KIAA0567, representing a gene of unknown function, had been isolated from a brain cDNA library. We determined the genomic structure of the underlying gene, which later turned out to be OPA1, and which is split into 29 exons covering about 100 kb of the genomic sequence.

The OPA1-GTPase

Examination of the N-terminal leader sequence of the deduced protein revealed the typical features of a protein imported into the matrix space of mitochondria. This is based on 1) an enrichment of basically charged aminoacids and 2) the presence of the MPP/MIP cleavage consensus sequence RX(F/L/I)XX(G/S/T)XXXX. OPA1 shows homology to dynamin-related large GTPases from salmon, C.elegans, Drosophila and the rat. The GTPase domain, encompassing the core central region between amino acid residues 280-520, harbours the consensus tripartite GTP binding motif needed for phosphate binding (GXXXXGKS/T), coordination of Mg2+ (DXXG), nucleotide binding (T/NKXD), and the dynamin sequence signature which are characteristically conserved in dynamin-related GTPases. The C-terminus of OPA1 differs from other dynamin family members in that it lacks a proline-rich region, a GED domain and a pleckstrin homology domain and may determine the specific functions of the OPA1 protein. Studies in yeast have demonstrated that the dynamin-related large GTPases Dnm1, MGM1, and MSP1 play an important role in the maintenance and inheritance of mitochondria.

Expression analysis by Northern blot hybridisations showed that OPA1 was 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 hypothetical pathophysiology of ADOA.



Protein domains od Dynamin and Dynamin-related GTPases
Apart from the GTPase domain, which is highly conserved between the Dynamin-related proteins, OPA1, MSP1 and MGM1 contain a mitochondrial leader sequence at their N-terminus

The distribution of OPA1 mutations

Extensive mutation screening in panels of patients from the UK, Denmark, and Germany provides the first indications on the distribution of mutations in ADOA patients. Frameshift-causing insertions and deletions account for the majority of the different mutations, followed by missense mutations, splice site mutations, stop mutations and in-frame deletions. The identified mutations are not evenly distributed along the length of the OPA1 polypeptide. There is a significant clustering of more than half of the different mutations between residues 270 and 468. Interestingly, this segment which corresponds to the putative GTPase domain of the OPA1 protein includes most of the identified missense mutations. Sequence

variations affecting the binding of GTP or GTP hydrolysis seem not to be tolerated and result in disease symptoms. Moreover, minor mutation clusters have been observed in the N-terminal leader sequence, as well as in the C-terminus of the OPA1 protein.

Identification of a founder effect in the Danish population

An important discovery was the identification of a founder mutation in about 50% of the Danish ADOA patients, which could explain the higher incidence of the disease in Denmark compared with other countries. The founder mutation is located in the last coding exon of the OPA1 gene, exon 28.

Selected Publications

Alexander, C., Votruba, M., Pesch U.E.A., Thiselton, D.L., Mayer, S., Moore, A., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G., Bhattacharya, S.S., 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.

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Thiselton, D.L., Alexander, C., Morris, A., Brooks, S., Rosenberg, T., Eiberg, H., Kjer, B., Kjer, P., Bhattacharya, S.S., Votruba, M. (2001). A frameshift mutation in exon 28 of the OPA1 gene explains the high prevalence of dominant optic atrophy in the Danish population: evidence for a founder effect. Hum. Genet. 109 (5): 498-502

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Neuronal Stem Cells

Gerd Kempermann

Huge numbers of new neurons are generated in the adult brain. Neuronal stem cells, from which new neurons can be derived, have been found throughout the entire brain. However, only two "privileged" regions of the adult brain make use of this potential and produce new neurons throughout life in any meaningful number. An interesting question is: why is this so? And why is the brain so poor at regeneration and why does it not make use of its potential for regeneration? What makes a neurogenic region neurogenic? Why do we have stem cells in the adult brain and what are they actually for?

Besides their biological significance for normal brain function, neuronal or neural stem cells have a great potential as a novel therapeutic tool. They are associated with none of the ethical problems that hamper the use of embryonic stem cells, they can be obtained from a patient himself for autologous transplantation, and they are pre-determined for a neuronal lineage. Could we use the knowledge about the regulation of adult neurogenesis in vivo to induce targeted neurogenesis in order to achieve neuroregeneration without the necessity of transplantation? In addition, stem cells might themselves play an important role in normal and pathological processes in the adult brain, most of which still remain to be identified.

Activity-dependent regulation of adult hippocampal neurogenesis

The main focus of our research group is the study of neuronal stem cells in their normal microenvironment within the neurogenic region of the adult hippocampus. We have shown that adult hippocampal neurogenesis is regulated in an activity-dependent manner and may play a role in learning processes. For example, physical activity robustly induces cell proliferation in the hippocampus. Experience of a complex environment increases the survival of newborn neurons. These paradigms provide a straightforward model system to address different aspects of neuronal stem cell biology in vivo.

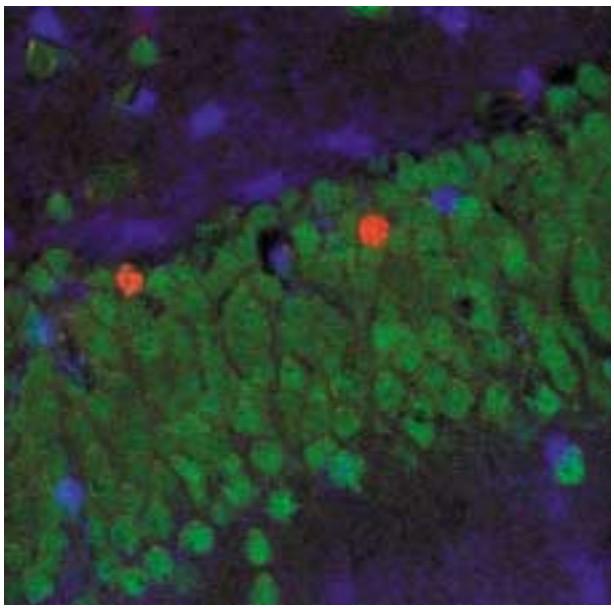
What makes a neurogenic region neurogenic? How is adult hippocampal neurogenesis regulated?

Current projects include the interaction of circadian rhythm with the activity-dependent regulation of adult hippocampal neurogenesis, the control of cell cycle parameters in the proliferating cells in the subgranular zone, and a detailed anatomical study of the interaction between neuronal stem cells and their microenvironment within the subgranular zone. In this context, we are also particularly interested in identifying key gene loci that correlate with the regulation of adult neurogenesis.
We are also studying the proliferative activity in non-neurogenic regions (e.g. the neocortex). Here, we are investigating how factors with known effects on neuronal stem cells in the hippocampus can influence proliferative activity and cell genesis in regions where, at least under normal conditions, no adult neurogenesis occurs.

How do neuronal stem cells contribute to brain function in health and disease?

To address this issue, we are focusing on the potential contribution of adult hippocampal neurogenesis to learning processes. Current projects are investigating a correlation and potentially causal link between performance in learning tasks, adult hippocampal neurogenesis and the genetic background. In a cooperative project, we are trying to develop a theoretical model of hippocampal function that incorporates the activity of neuronal stem cells in the adult hippocampus. A role of neuronal stem cells in normal brain function may also imply that an impaired regulation of stem cell activity in the adult brain could contribute to the pathogenesis of neurological disorders. Here, we are particularly interested in the role of stem cells in major depression and brain tumors.

Two new nerve cells in the adult mouse brain. They are made visible by immunofluorescence, a method by which specific cell types can be identified under the microscope. Here, the two new cells appear orange, because they carry two different markers that identify them both as a newly generated cell and a neuron.



The research group “Neuronal stem cells” works in close interaction with the independent group “Neurogenic permissiveness”, also headed by Gerd Kempermann, funded by Volkswagen Stiftung and located at the Dept. of Neurology, Charité University-Hospital, Berlin. The goal of this interaction is to allow clinicians to pursue stem cell research involving close interaction with the clinic, but within the scientific environment provided by the MDC.

Selected Publications

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van Praag, H., Kempermann, G., and Gage, F.H. (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature Neuroscience 2, 266-270.

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Cellular Neurosciences

Helmut Kettenmann

The central nervous system contains two major cell populations, neurons and glial cells. The neurons are regarded as the elements mediating the electrical activity in the brain. As a consequence, neuroscience research in 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, recent technical advances brought these cells into the neuroscience arena. It is now evident that glial cells are essential for the proper functioning of the brain and different types of glial cells fulfill distinct tasks. Oligodendrocytes are the myelin-forming cells of the central nervous system which ensure 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 the group has previously studied aspects related to all three types of glial cells, the present research program is now focussed 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. Each of these topics is intergrated in and funded by a particular group grant from the German Research Council (Sonderforschungsbereich 507, 515, Schwerpunktprogramm on Microglia).

1. How do astrocytes detect neuronal activity ?

In recent 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 have found that the activity of parallel fibers, the axons of the granule cells synapsing onto Purkinje neurons, triggers a calcium signal in Bergmann glial cells. At a moderate level of activity, the signal can be confined to a subregion of the cell which has a morphological correlate, the microdomain. Following more intense stimulation, the signal spreads to the soma. This form of neuron-glia interaction is mediated by NO which is known to be released from parallel fibers. We believe that these units could feedback information on a defined population of synapses, namely those which are enwrapped by a given microdomain.

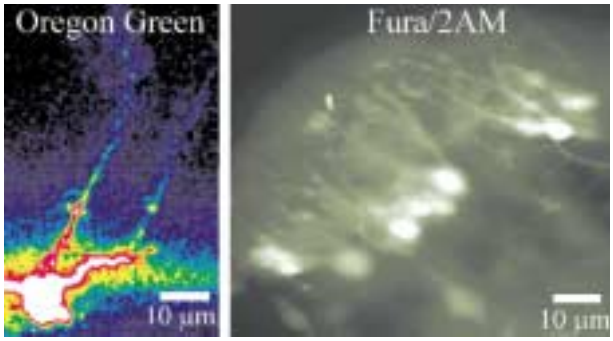
2. How do astrocytes communicate with each other?

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. While calcium signals did not spread among Bergmann glial cells., we found a wide-spread activation in astrocytes from corpus callosum slices. The communication among 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 at a low speed, about 10 mm/s, and is thus 1 000 000 times slower than the neuronal action potential. We identified these astrocytes using a transgenic mouse in which all astrocytes were labelled with a fluorescent protein. This approach showed us that, unlike in culture and the retina, in brain tissue the glial calcium wave is more global, i. e. it also activates cells of the oligodendrocyte lineage and microglial cells.

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

Microglial cells are the major immunocompetent cells in the brain. 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 funtional receptors and physiological phenotype of these cells. Using this approach we have recently reported that resting microglia express a physiological phenotype which is distinct from all other CNS cell types and also differs from macrophages. Interestingly microglial cells in situ express different types of purinergic receptors and so are suitable receptive elements to detect astrocyte activity mediated by calcium waves.

Stimulation of parallel fibers triggered calcium responses in somata and processes of Bergmann glia cells. To record responses in processes, cells were perfused via a patch pipet with Oregon green BAPTA-1 (left). The soma (white spot) and two processes can be recognized in this color-coded micrograph. On the right, a slice with bulk-loaded (Fura-2/AM) Bergmann glial cells is depicted.
From Matyash V, Filippov V, Mohrhagen K, Kettenmann H. Nitric oxide signals parallel fiber activity to Bergmann glial cells in the mouse cerebellar slice. (2001) Mol Cell Neurosci. 18:664-70.



4. What are the factors controlling the properties of astrocytes in pathological conditions?

Astrocytes respond with reactive gliosis to brain injury indicated by an increase in proliferation and migration to the lesion site. To study factors controlling astrocyte migration, we have examined how cultured astrocytes migrate into a cell-free zone produced by a scratch within a cell monolayer. Blocking ryanodine receptors, intracellular calcium-release channels, strongly attenuated the migratory activity of astrocytes. RT-PCR analysis, Western blotting and immunocytochemistry revealed that cultured and acutely isolated astrocytes express exclusively the ryanodine receptor 3 isotype (RyR3). In cultures prepared from RyR3-deficient mice, astrocyte motility was significantly impaired and ryanodine did not induce any further decrease in the rate of cell migration. These results indicate that calcium signaling via RyR3 is an important factor for the control of astrocyte migration.

5. What are the factors controlling the properties of microglial cells in pathological conditions?

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

6. What are the physiological properties of gliomas and how do they compare with 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 are studying the cellular properties of these tumor cells and comparing them with normal glial cells with respect to their physiological properties, their ability to proliferate and to migrate. Recently, we focused on the expression of GABA receptors by glioma cells since we have found a very strong correlation between the expression pattern and the malignancy of the tumor. Only cells from tumors with low malignancy express GABA receptors, and all tumor cell lines which are selected for high proliferative activity lack this receptor. We have evidence that contact with neurons triggers the induction of this receptor and the expression is correlated with an decrease in metabolic activity.

Selected Publications

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Lyons, S. A., Pastor, A., Kann, O., Ohlemeyer, C., Wiegand, F., Prass, K., Knapp, F., Kettenmann, H. and Dirnagl U. (2000) Distinct physiological properties of microglia and blood-borne cells in rat brain slices after permanent middle cerebral artery occlusion. J. Cer. Blood Flow Met., 20:1537-1549.

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Schipke C., Ohlemeyer C., Matyash M., Nolte C., Kettenmann H., and Kirchhoff F. (2001). Astrocytes of the mouse cortex express functional N-methyl-D-aspartate receptors, FASEB J. 15, 1270-1272

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Growth Factor and Regeneration Group

Gary R. Lewin

Sensory neurons of the dorsal root ganglia allow us detect stimuli to the body surface that lead directly to 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 are also studying the genetic programs controlled by growth factors that specify sensory neuron function or their connections to spinal cord circuits.

Ongoing Projects

Molecular Basis of Mechanotransduction

Mechanotransduction is the process whereby receptor proteins present in the endings of sensory neurons are able to detect mechanical stimulation of the tissue they innervate. We have used information from genetic experiments in the simple nematode worm C.elegans to identify possible vertebrate candidate proteins that might detect mechanical stimuli. Worms have been developed with gene mutations that made them insensitive to touch. Some of these genes encoded membrane ion channels that were proposed to affect movement or displacement of the plasma membrane. We have recently shown that a mouse protein (BNC1), 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 ion channel works in concert with other ion channel subunits (eg. the DRASIC protein) to detect mechanical forces (Price et al., 2001). In addition to the ion channel other work in C.elegans has identified a membrane protein called Mec-2 that might interact and regulate the activity of the channel. We have cloned new vertebrates homologues of this gene and are presently characterizing their in vivo function using mouse genetics in combination with electrophysiology (for methods used see Hamilton et al. 2001; Stucky and Lewin, 1999). In the longer term, we hope to identify the exact molecular complexes that allow sensory neurons to detect mechanical forces. We hope that heterogeneity in the molecular complexes involved might explain the diversity of modalities that different kinds of sensory neurons are able to detect.

Regulation of sensory synaptic connections in the spinal cord

The synaptic connections made by sensory neurons in the spinal cord are the basis for 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). We are presently developing this technique to provide high quality phenotype information on many different mouse mutants to identify genes regulating the construction or function of spinal reflexes. 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.

Selected Publications

Price M.P., McIlwrath S.L., Xie J., Cheng C., Qiao J., Tarr D.E., Sluka, K.A., Brennan, T.J., Lewin G.R., Welsh M.J. (2001) The DRASIC cation channel contributes to the detection of touch and acid stimuli in mice. Neuron 32, 1071-1083.

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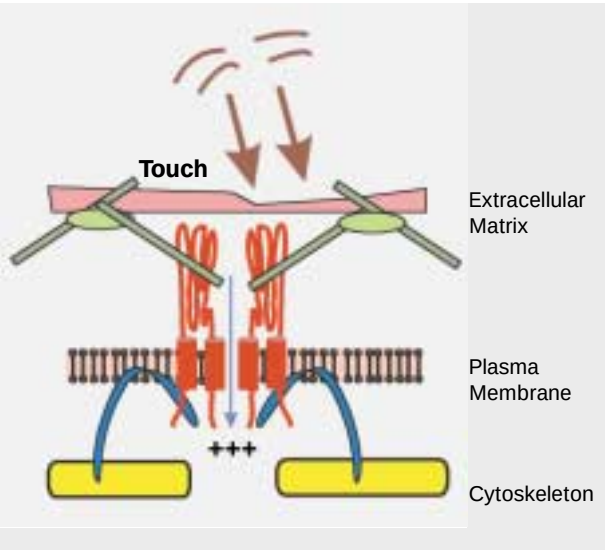
Hamilton S.G., McMahon S.B. and Lewin G. R (2001) Selective activation of nociceptors by P2X receptor agonists in normal and inflamed skin. Journal of Physiology 534(2): 437-445.

Model of how a complex of proteins might detect a mechanical force on the cell membrane on a sensory neuron ending. Ion channels in the plasma membrane that conduct an inward current carried mostly by sodium ions are linked to the cytoskeleton and to the extracellular matrix. Mechanical forces open the channel as shear forces are created by its attachment to relatively stiff cytoskeletal and extracellular matrices.



Price M.P., Lewin G.R., McIlwrath S.L., Cheng C., Xie J., Heppenstall P.A., Stucky C.L., Mannsfeldt A.G., Brennan T.J., Drummond H.A., Qiao J., Benson C.J., Tarr D.E., Hrstka R.F., Yang B. Williamson R.A., Welsh M.J. (2000) The Mammalian Na+ Channel BNC1 Is Required for Normal Touch Sensation. Nature 407:1007-11

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Synapse Formation and Function

Frank W. Pfrieger

A major aim of neurobiological research is to understand the formation and function of chemical synapses, highly specialized intercellular connections that mediate the exchange of electrical signals between neurons. Our group focuses on the identification of the signals and mechanisms that control the development of synapses in the mammalian central nervous system (CNS).

Identification of cholesterol as a glia-derived factor promoting synapse development in CNS neurons.

The top priority of our group has been to identify a glia-derived factor that promotes synapse development in retinal ganglion cells (RGCs) and to analyse its mode of action. The identification of this factor has been long-awaited, ever since the publication by Pfrieger & Barres (1997) and there has been increased interest due to recent publications (Nägler et al., 2001; Ullian et al. 2001). Recently, we succeeded in identifying the factor by the use of two parallel experimental approaches. Firstly, we partially purified the component from glia-conditioned medium by a series of chromatographic separation steps using electrophysiological recordings of synaptic activity as a bioassay. Secondly, we detected glia-induced changes in the protein composition of neuronal membranes by two-dimensional gel electrophoresis and mass spectrometry. Surprisingly, we found that cholesterol released by glial cells in apolipoprotein E-containing lipoproteins completely mimicked the effects of glia-conditioned medium on synapse development (Mauch et al., 2001).

Since cholesterol homeostasis in the brain is independent of blood levels, our results suggest the following scenario: RGCs appear to produce enough cholesterol to support their vital cellular functions and form axons and dendrites, but require large additional amounts to develop numerous and mature synaptic connections. Consequently, they depend on cholesterol synthesis by glial cells and its delivery via apolipoprotein E-containing lipoproteins. These findings have two important implications: firstly, they suggest a new role for glial cells that may explain, why massive synaptogenesis in the CNS occurs just after differentiation of macroglial cells, and why glial cells secrete cholesterol-rich lipoproteins at all; secondly, they provide the first evidence for an exciting link between cholesterol homeostasis and the development and plasticity of synaptic connections, which may help to explain some of the neurologic symptoms resulting from genetic defects in cholesterol or lipoprotein metabolism.

A new method to isolate CNS neurons

The development of neurons is controlled by extrinsic signals from non-neuronal cells, particularly from glia. One possible way to analyse the underlying signaling pathways is to separate neurons from these cells and to monitor their development in culture under strictly defined conditions. However, this elegant approach has been hampered by the lack of suitable methods to isolate CNS neurons. So far, only RGCs have been separated from other cells in sufficiently high numbers. In this project, we aimed to establish a new method to obtain highly purified neurons from postnatal mouse hippocampus and to culture these under strictly defined conditions in the absence of non-neuronal cells (Nägler & Pfrieger, submitted). We found that mouse hippocampal neurons can be isolated in 99.9% purity by a modified version of the immunopanning protocol that can be used to isolate RGCs. This new cell culture preparation will allow us, for the first time, to define the fundamental survival and differentiation requirements of these key CNS neurons.

Selected Publications

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Developmental Neurobiology

Fritz G. Rathjen

Activity-dependent and –independent processes regulate the pattern of neuronal connectivity

The formation of precise and selective synaptic connections between neurons is essential for the proper functioning of the nervous system. The establishment of these synaptic connections throughout the nervous system involves a complex series of events. During embryonic development growth cones, the tips of extending axons, respond to an array of molecular signals that are present in their microenvironment. These distinct signals are mediated and integrated by so-called guidance receptors on the surface of growth cones to activate intracellular signal transduction pathways that modulate the actin-myosin cytoskeleton. Several classes of guidance receptors have been shown to be implicated in these processes including neural members of the immunoglobulin superfamily (IgSF), semaphorins, netrins, ephrins, neuropilins, plexins, Eph-kinases and several extracellular matrix proteins.

Once the initial pattern of neuronal connectivity has been established by axonal guidance receptors, these synaptic connections are subsequently refined by processes dependent on the electric activity of neurons. It is likely that simple behaviours such as reflexes are mediated by neuronal circuits that are completely formed in animals without any need of neuronal activity and will not be re-shaped. In contrast, circuits that are responsible for more complex behaviours, including those that are essentially human, might be influenced to a substantial degree by experience-dependent activity. Experience, as soon as it is transduced into neuronal action potentials, appears to modulate the wiring of the nervous system to suit the unique needs of its owner. It is, therefore, a fascinating question of how epigenetic influences, such as neuronal activity, interact with genetic instructions to form and modify circuits within the nervous system.

Axonal IgSF members

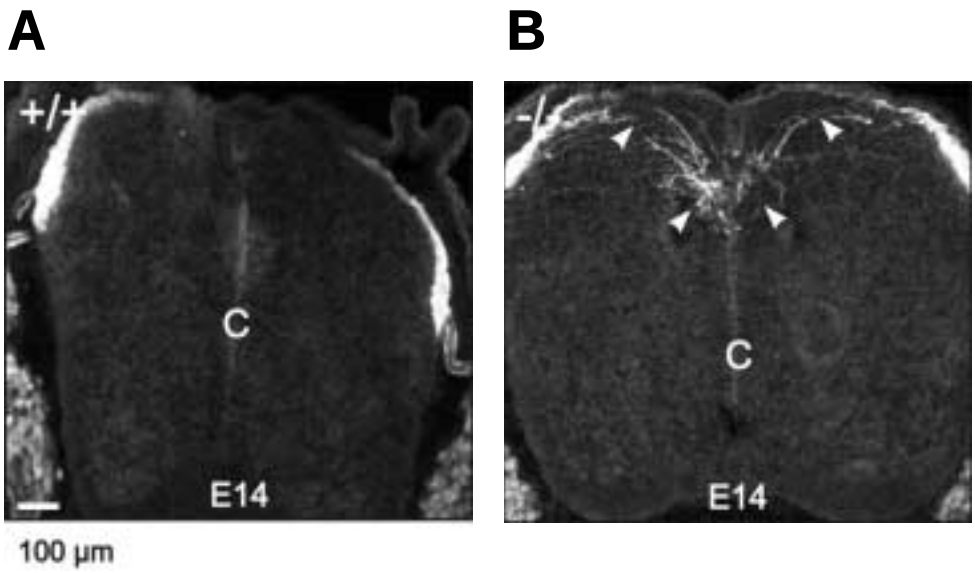
The most diverse class of proteins that is implicated in contact-dependent regulation of axon guidance are the neural members of the IgSF which can be categorized into several structural subclasses including the L1- and F11-subgroups. Functional in vitro studies and intriguing in vivo observations in chicks, mice and in humans have indicated that these proteins are indeed important for the correct wiring of the nervous system. Our research in the past grant period on this class of proteins has focussed on their intracellular sorting, disease models and the identification of novel members.

The L1 protein, which is the first member of a subfamily of the IgSF (L1, neurofascin, NrCAM and CHL1), has been demonstrated to be involved in hereditary brain disorder with a relatively broad spectrum of symptoms. It has been termed X-linked hydrocephalus, MASA syndrome or spastic paraplegia type I and includes mental retardation, hydrocephalus, hypoplasia of the corticalspinal tract and an underdeveloped corpus callosum. As a first step towards an understanding of the molecular mechanisms of these diseases, we investigated how mutations in the L1 gene influence binding of different ligands and intracellular trafficking of the L1 protein (in collaboration with S. Kenwrick, Cambridge). These studies showed that different disease-associated mutations have distinct effects on heterophilic ligand binding as well as on the expression of L1 on the cell surface. Our results suggest that some aspects of patient pathology are due to disturbances in interneuronal interactions.

To study the function of NrCAM, a member of the L1 subfamily, in an in vivo situation we generated and analyzed a mouse strain deficient for this gene. We found that the absence of NrCAM causes the formation of mature cataracts in the mouse. Cataracts, the most common cause of visual impairment, are generated in NrCAM-deficient mice by a disorganization of lens fibers, followed by cellular disintegration and accumulation of cellular debris. The disorganization of lens fiber cells includes abnormalities of the cytoskeleton and connexin50-containing gap junctions.

To identify proteins that interact with L1 subfamily members intracellularly and therefore might link L1 members to trafficking, spatial targeting or signalling pathways we used yeast two-hybrid screening. We have demonstrated that syntenin-1, an intracellular protein containing two PDZ domains, interacts specifically with the cytoplasmic segment of neurofascin but not with other L1-subfamily members. Although the biological function of this interaction remains to be defined it is conceivable that syntenin-1 might function by linking bound neurofascin to trafficking or recycling pathways in neural cells.

By a PCR-based screen we identified FAR-2, a novel F11/F3/contactin-related protein of the IgSF that is expressed by subsets of cerebellar Purkinje cells, predominantly in the caudal part of the chick cerebellum. FAR-2-positive clusters of Purkinje cells alternate with FAR-2-negative clusters in both tangential dimensions of the cerebellar cortex, along the rostrocaudal axis and along the mediolateral axis. The patchwork-like expression pattern of FAR-2 in the cerebellar cortex, in combination with its structural similarity to



cGMP signalling via cGKIα is important for the correct pathfinding of sensory axons. The pathfinding of sensory axons within the developing spinal cord was analyzed. Nociceptive axons in (A) wild-type (+/+) and in (B) cGKI-deficient mice (-/-) of transverse sections of the spinal cord. In the absence of cGKI sensory axons leave the dorsal funiculus prematurely and grow towards the central canal (arrowheads). C, central canal; E14, embryonic day 14.

F11, suggests that it may contribute to the formation of fractured somatotopic maps of cerebellar afferents during embryonic development.

Signaltransduction systems of growth cones activated for axonal pathfinding

Several membrane proteins are known to mediate axon guidance during embryonic development of the nervous system. The intracellular signal cascades activated by these guidance receptors and how distinct signals are integrated to re-organize the cytoskeleton of the growth cone are less well defined. Recently, Poo and co-workers (UCSD) showed by an in vitro growth cone turning assay that cyclic nucleotides modulate the signal transduction system within extending axons. The intracellular components activated by cGMP, however, remain unknown. In many cell types cGMP is a widely used second messenger that has several targets including cGMP-dependent protein kinase I (cGKI). By using embryonic dorsal root ganglia (DRG) from cGKI-deficient mice, we have shown that activation of cGKI counteracts semaphorin 3A-induced growth cone collapse in vitro (in collaboration with F. Hofmann, München). Since the α isoform of cGKI was found to be selectively expressed in sensory axons during development we analyzed the trajectories of these axons in cGKI-deficient mice (figure). These studies demonstrate that cGKI-deficient mice have a longitudinal axon guidance defect of sensory axons within the dorsal root entry zone (DREZ). Consequently, less axons grow within the primordium of the dorsal funiculus of the spinal cord in cGKI-deficient mice. Our current studies focus on downstream signalling targets of cGKI in sensory growth cones.

Screens to identify cell surface proteins modulated by neuronal activity

Although in the past decade an increasing number of cell surface glycoproteins known to act as guidance cues have been identified, much less is known about components important for the regulation of synapse formation. In addition, despite compelling evidence that experience-dependent electrical activity modulates neuronal circuits in several systems of the brain, the molecular components mediating these processes are largely unknown. Therefore, in recent years, we have been interested in identifying proteins which are modulated by neuronal activity. Using specific labeling procedures, we have concentrated our screening on cell surface proteins since these might function as receptors or ligands which mediate structural changes within the nervous system. Cell surface proteins modulated by electrical activity are considered as candidates implicated in the establishment of synapses and important for structural plasticity in the brain.

Up to now, we have identified two proteins modulated on the surface of neurons by activity-dependent processes, one being up-regulated while the other is down-regulated. Our current efforts concentrate on the functional characterization of these proteins using genetic mouse model systems.

Publications 2001

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Structure of the Group

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Dr. René Jüttner
Dr. Michael Koroll
Dr. Margret Moré[2)]
Dr. Ursel Nörenberg[1)]
Dr. Ute Zacharias

1) deceased
2) maternity leave

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Secretariat
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* until 11. 2001

Proteomics and Molecular Mechanisms of Neurodegenerative Disorders

Erich Wanker

The accumulation of highly insoluble intra- and extracellular protein aggregates is a common feature of progressive late-onset neurodegenerative disorders including Alzheimer's, Parkinson's, and Huntington's disease. Although the causal relationship between protein aggregation and neurodegeneration has not been proven, within recent years several lines of biochemical and genetic evidence have suggested that the process of aggregate formation in patient brains is linked to neuronal dysfunction and disease progression. The main objective of our work is to understand the pathophysiological mechanisms of late-onset neurodegenerative disorders and to develop causal therapies. Another goal of our research is to understand the normal function of proteins involved in neurodegenerative disorders. We use high-throughput functional genomics approaches for the identification of large protein-protein networks and novel drug targets. Furthermore, *in vitro* and *in vivo* functional assays are being developed for high-throughput drug screening.

Polyglutamine aggregation in Huntington's disease

Huntington's disease (HD) is caused by an elongated polyglutamine (polyQ) sequence within a large protein, huntingtin, of unknown function. We have found that N-terminal huntingtin fragments with polyQ tracts in the pathological range (>37 glutamines), but not with polyQ tracts in the normal range (20-30 glutamines) form high molecular weight protein aggregates with a fibrillar morphology. The self-assembly of huntingtin fibrils *in vitro* depends highly on the polyQ repeat length, protein concentration and time. Furthermore, aggregate formation can be seeded by preformed fibrils, suggesting that huntingtin protein aggregates in neuronal cells are formed by a nucleation-dependent process. To study huntingtin aggregation *in vivo*, a cell culture model system of HD has been developed. In mammalian cells, N-terminal huntingtin fragments with polyQ tracts in the pathological range (51 or 83 glutamines) accumulated in aggresome-like perinuclear inclusion bodies. Interestingly, inhibition of proteasome activity with lactacystin resulted in a twofold increase in the amount of ubiquitinated huntingtin aggregates, indicating that the activity of the ubiquitin-proteasome system is critical for the accumulation of mutant huntingtin protein *in vivo*. Moreover, immunofluorescence and electron microscopy revealed that the 20S, 19S and 11S subunits of the 26S proteasome co-localize with the perinuclear inclusions. Thus, our findings support the hypothesis that the ubiquitin-proteasome system is a potential target for therapeutic interventions in HD and related glutamine repeat disorders.

Identification of polyglutamine aggregation inhibitors by high-throughput screening

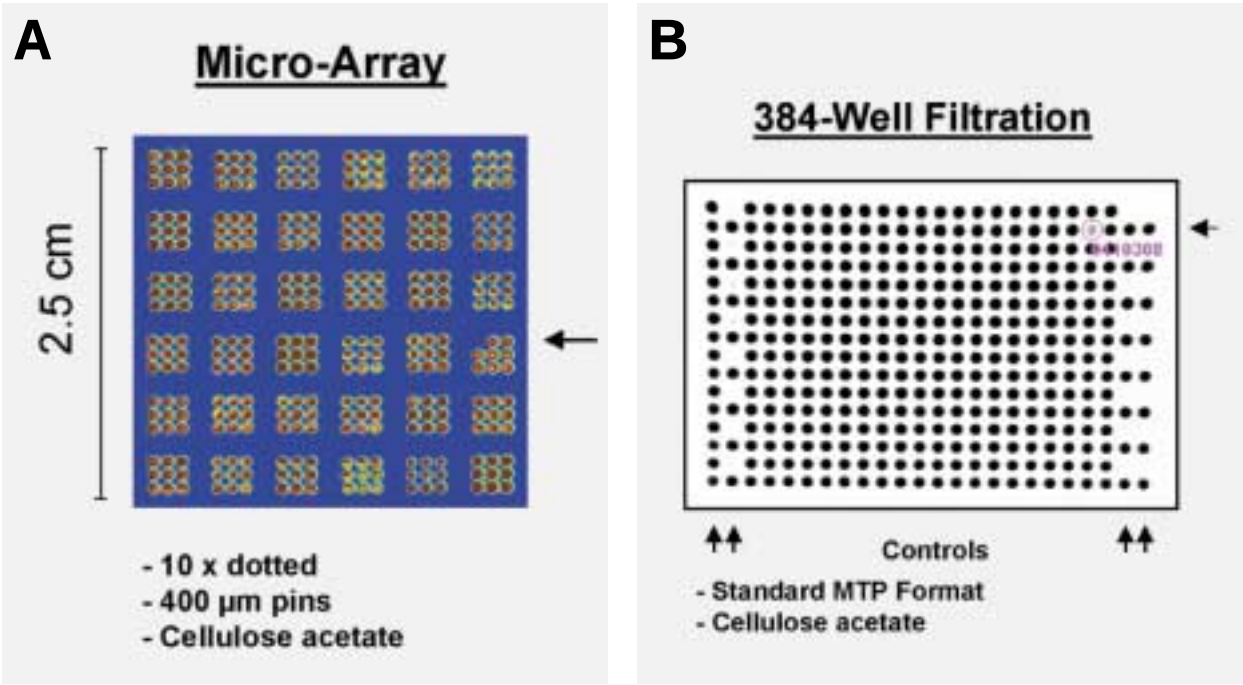
We suggest that the process of protein aggregation is a key step in the development of neurodegenerative disorders. Thus, preventing aggregate formation must slow down disease progression. In order to identify huntingtin aggregation inhibitors, we have developed a sensitive filter retardation assay which is suitable for high-throughput drug screening. Using this assay we first tested a number of known inhibitors of β-amyloid, PrP^{scr}, and microtubule fibril formation for their effect on huntingtin aggregation. We found that Congo red, thioflavine S, chrysamine G and Direct fast yellow are potent inhibitors of polyQ aggregation and suppress the self-assembly of HD exon 1 protein in a dose-dependent manner. These results were confirmed in cell culture models of HD and we also showed that the monoclonal antibody 1C2, which specifically recognizes the elongated polyQ stretch, is a potent inhibitor of huntingtin aggregation. Using an automated filter retardation assay, we tested ~184,000 chemical compounds and identified about 700 small molecules that prevent huntingtin aggregation *in vitro*. Currently, these compounds are being tested in cell culture model systems of HD.

Chaperones are potent inhibitors of protein aggregation in neurodegenerative disorders

It is well known that molecular chaperones prevent misfolding and aggregation of disease proteins. We have found that a combination of the heat shock proteins Hsp70 and Hsp40 is most effective in inhibiting huntingtin aggregation *in vitro* and in mammalian cell culture model systems. In addition, treatment of mammalian cells with geldanamycin, a naturally occuring antitumor drug, induced the expression of Hsp70 and Hsp40 and blocked huntingtin aggregation in a dose-dependent manner. This was the first demonstration that deposition of mutant huntingtin in cells can be suppressed by chemical compounds activating a specific heat shock response. Our findings may provide the basis for the development of a novel pharmacotherapy for HD and related glutamine repeat disorders.

Analysis of huntingtin function and dysfunction

In order to understand huntingtin function we have searched for interacting proteins using the yeast two-hybrid system. We have found that the proteins HIP1 and SH3GL3 specifically interact with huntingtin. HIP1 is homologous to the yeast protein Sla2p, which is associated with the membrane cytoskele-



Identification of chemical compounds as potential polyglutamine aggregation inhibitors of Huntington's disease using an automated filter retardation assay. GST-HD51 fusion protein was predigested with protease and incubated for an additional 16 h at 37°C in the presence of various chemical compounds. Then, aggregation reactions were transferred to a cellulose acetate membrane using a spotting robot (A) or filtered through a cellulose acetate membrane using a 384-well dot blot apparatus (B). Captured aggregates were detected by immunoblotting using an anti-huntingtin antibody. A total of 320 different chemical compounds were tested on each filter membrane. The arrow marks an inhibitory compound identified by image analysis.

ton and plays a functional role in endocytosis. Thus, both HIP1 and huntingtin may also function in this process in higher eukaryotes. To test this hypothesis, we screened for HIP1-interacting proteins using affinity chromatography and mass spectrometry. We found that HIP1, in addition to huntingtin, also associates with clathrin heavy chain and α-adaptin A and C. Furthermore, HIP1 function is critical for receptor- mediated endocytosis in mammalian cells. Based on these studies, we propose that HIP1 and huntingtin are involved in the recruitment of clathrin coats to lipid membranes. However, additional studies will be necessary to address this question in more detail.

Identification of protein-protein interaction networks by automated two-hybrid screening

As the human genome is unraveled, research focus in the future will shift to the functional analysis of gene products. In order to identify protein-protein interactions on a large scale we have developed an automated yeast two-hybrid system. We use interaction mating to generate large arrays of yeast clones containing protein-protein interactions. Furthermore, pipetting, picking and spotting robots are used for the parallel handling of large numbers of yeast clones. In the last year we have applied the automated two-hybrid system for the identification of protein-protein interactions involved in HD. Using this method 24 novel protein-protein interactions were found. Protein interactions were verified by *in vitro* binding experiments, co-immunoprecipitations and co-localization studies. Currently, the automated yeast two-hybrid system is being used to identify the partner proteins of ~1000 human disease proteins. We believe that understanding the protein-protein networks of human diseases will help to identify novel drug targets for therapeutic intervention.

High-throughput protein expression and functional assays

In our laboratory systematic efforts are currently underway to construct defined sets of cloned human genes for high-throughput expression and purification of recombinant proteins. Purified recombinant proteins are valuable resources for many applications in functional genomics and protemics. For example, proteins are the basic reagents for the production of protein-chips or for studying protein structure. Furthermore, they are used in affinity chromatography-based methods to identify protein-protein interactions. During the last two years a method for the high-throughput identification of protein complexes by affinity chromatography and mass spectrometry has been developed in our laboratory. We have used this method for the identification and characterization of protein-protein interactions that play a key role in neurodegenerative disorders and signal transduction. We propose that the large scale application of affinity chromatography-based methods will allow the functional characterization of unknown proteins. In addition, it will contribute to the understanding of disease processes.

Selected Publications

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Erika Pisch

Structure and Organization

Organizational Structure

As provided by §7 of its charter the organizational structure of the Max Delbrück Center for Molecular Medicine (MDC) are the following:

• the Board of Trustees with the Scientific Committee,
• the Management Board.

The Board of Trustees

As provided by § 8 of the Charter of the Foundation, the Board of Trustees ensures that the transactions of the Foundation are conducted in a lawful, expedient and financially responsible manner. The Board determines within the framework of the law the broad research objectives and the main research policy and financial matters of the Foundation, lays down principles of management and the principles for evaluating results, intervenes appropriately within the decisions of the Board of Management, and directs the Board of Management in special matters of research policy and finances.

Furthermore, the Board of Trustees approves annual and extended budgets (including expansion and investment programs), draws up the Charter and decides upon amendments to it, decides upon the dissolution of the Foundation, and takes decisions in other cases provided for in the Law and the Charter.

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Prof. Dr. Hans Meyer
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Prof. Dr. Ihre Königliche Hoheit Prinzessin Chulabhorn Mahidol, Präsidentin des Chulabhorn Forschungsinstitut in Bangkok (Thailand) besuchte das MDC und den Campus Berlin-Buch am 29. September 2000
Prof. Dr. Her Royal Highness Princess Chulabhorn Mahidol, President of the Chulabhorn Research Institute in Bangkok (Thailand) visited the MDC and the Berlin-Buch Campus on September 29, 2000
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Max Planck Institute for Biophysical Chemistry, Göttingen

Prof. Dr. Lennart Philipson
Karolinska Institut, Stockholm, Sweden

Grundsteinlegung für ein Laborgebäude des Biotechnologieparks mit den damaligen Berliner Senatorinnen Juliane Freifrau von Friesen (3. v. l.) und Gabriele Schöttler (2. v. l.) am 12. Oktober 2001 mit der Bauherrin Dr. Gudrun Erzgräber von der BBB Management GmbH Campus Berlin-Buch.
Laying the foundation stone for yet another new laboratory building in the Biotechnology Park with the former Berlin Senators Juliane Freifrau von Friesen (3rd from left) and Gabriele Schöttler (2nd from left) on October 12, 2001, as Dr. Gudrun Erzgräber (left), BBB Management GmbH, Campus Berlin-Buch, looks on.
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Prof. Dr. Kai Simons
Max Planck Institute for Molecular Cell Biology and Genetics, Dresden

Prof. Dr. Alex J. van der Eb
Departement of Molecular Carcinogeneses, Sylvius Laboratories, Leiden, The Netherlands

The Management Board

As provided by §13 of the Charter, the Management Board directs the Foundation. The Board of Management consists of one or more scientific members and one administrative member, and is chaired by a scientific member. The Board of Management is appointed by the Board of Trustees. Since January 1, 1992, the chairman and scientific member has been Prof. Detlev Ganten. Dr. Erwin Jost was the administrative member of the Management Board until December 2001, and was succeeded by Dr. Waltraud Kreutz-Gers in January 2002. Prof. Dr. Walter Birchmeier has been a scientific member of the Management Board and deputy scientific director.

Scientific Council

According to §14 of the Charter of the MDC, the Scientific Council advices the Management Board in matters of fundamental scientific importance. The Scientific Council is consulted in all scientific appointments and formulates suggestions for the development of new research programs of the MDC.

During the past two years the Scientific Council discussed extensively the reorganization of the Helmholtz Association of National Research Centers (HGF) and advised the Management Board particularly in matters concerning the involvement of the scientists in all major decision processes as well as their appropriate representation in the newly established committees of the HGF. In addition, the Scientific Council made recommendations on scientific-adequate standardized controlling procedures to be developed within the HGF.

Members of the Scientific Council (as of November 2001)

Walter Birchmeier
Thomas Blankenstein
Kurt Bommert
Iduna Fichtner
Hannelore Haase
Udo Heinemann
Uta Höpken
Uwe Karsten
Helmut Kettenmann
Gary Lewin
Martin Lipp (Chair)
Friedrich Luft
Margret Irmgardt Moré
Thomas Müller
Claus Scheidereit
Peter Schlag
Ruth Schmidt-Ullrich
Wolfgang Uckert
Gerd Wallukat

Staff Council

The Staff Council at the Max Delbrück Center for Molecular Medicine has a legal right to participate in all matters associated with the Center. This includes participating in decisions and collaborating in matters involving employment, salary agreements and scale as well as in resolving internal problems and participating in staff discussions.
In addition, the Staff Council participates in the Canteen Committee and the chairmanship of the MDC Works Safety Committee is taken by a member of the Staff Council.

In the context of the national "Association of Work and Staff Councils of Extra-university Research Institutes" (AGBR), Staff Council members take part in the following committees. "Questions of Principle", "Data Protection", "Work, Health and Environmental Protection" and "Staff and Compensation" as well as "Further Education and Training".

With direct collaboration by the Staff Council, other projects have been initiated in which scientists and technicians, depending on their previous experience, have the opportunity to acquire new techniques and skills in order to be able to work in new research fields being investigated at the Max Delbrück Center on the Berlin-Buch Campus. In collaboration with BBB GmbH (Biomedical Research Campus Berlin-Buch), these projects are financed by the Berlin Senate for Employment, Womens, Health and Social Affairs, and by the Berlin Senate for Science, Research and Culture with help from the European Social Fund (ESF).

Members of the Staff Council 2001

Marion Bimmler (Chair)
Lutz Else
Ingrid Grunewald
Dr. Brigitte Hoch
Frank-Peter Kirsch
Dr. Peter Konzer
Bernd Lemke
Werner Liebig
Jana Richter
Christel Westen
Dr. Reinhard Zeisig

Der Architekt Andreas Timmermann überreicht Dr. Gudrun Erzgräber (BBB Management GmbH Campus Berlin-Buch) den Schlüssel anlässlich der Eröffnung des Otto-Warburg-Hauses am 12. Oktober 2001. Mit den damaligen Berliner Senatoren (v. l.) Klaus Böger, Gabriele Schöttler und Juliane Freifrau von Friesen.
Handing over the key: Architect Andreas Timmermann and Dr. Gudrun Erzgräber (BBB Management GmbH Campus Berlin-Buch) at the opening of the Otto Warburg House on October 12, 2001. Also present: the former Berlin Senators (from left) Klaus Böger, Gabriele Schöttler and Juliane Freifrau von Friesen.

Supporting Divisions

Safety

The diversity and quality of scientific research carried out at the MDC requires adherence to a wide range of laws, regulations, guidelines, and standards. Measures to ensure not only the quality of the science, but also the safety of researchers include the new Working Protection Law, the Genetic Engineering Law, the Radiation Protection Decree, the Chemicals Law and the Waste Law. The Safety Group regularly inspects facilities and compiles internal operating instructions to prevent working accidents, work-related illness, and emergencies. Safety instructions for working groups, identification of possible sources of danger, as well as equipment testing and the training in safety procedures are all important functions of the safety engineers. The Committee for Working Safety at the MDC consists of scientically trained safety officers and specialists in technical matters and radiation protection. The Safety Group regularly discusses topical problems concerning health and safety at work in close cooperation with the medical officer and the staff council.

Head: Dr. Peter Stolley / Dr. Regina Möhring

Building Coordination Engineering and Reconstruction

Early in 2000, the foundation stone was laid for the Communications Center on the Berlin Buch campus. The building work was carried out as planned and it was opened on 29.11.2001 by the President of the Federal Republic of Germany, Johannes Rau, at an award ceremony for the German Future Prize, a prize presented for Technology and Innovation.

In 2000, approval was given for setting up new animal facilities and a Building for Theoretical Studies. Extensive preparation of the site was needed before building could start in 2000.

Construction is going ahead as planned and it is expected to be able to complete the work and open it to staff in 2003.

Renovation of the facade of the Max Delbrück Haus has been delayed because of poor weather during the fourth quarter of 2001. However, the MDC has ensured that work will continue through the winter months and it is hoped to complete the work in the first quarter of 2002.

In June 2000, thanks to the efforts of donors, the Max Delbrück Center saw the first discussions of the building plans for “Medical Genome Research”. This meeting covered aspects such as staffing levels, number of research groups, and opinions about the infrastructure. The discussion were so fruitful that preliminary preparations can be started in January 2002 to establish the budgeting and accounting building. The building plans were prepared in collaboration with the FMP.

Head: Grit Kuhlmann

Auditing and Legal Affairs

The duties of Auditing and Legal Affairs are to plan and implement the inspection of and adherence to legal issues. The Auditing Office bears the responsibility for overseeing that laws, practices, regulations and the directives of the Management Board are properly observed and that allocated public resources are appropriately, economically and productively used. For these purposes, examination plans, approved by the Management Board, are instituted yearly to check regulatory compliance with organizational activities, so that individual actions are inspected on a case-by-case basis for full compliance with regard to both form and content.

All checks of regulatory compliance are undertaken with respect to economic feasibility and efficiency so as to ensure that proper judgment is exercised in establishing regulations and in the execution of all business matters. These checks result in the establishment of recommendations as to how detected oversights might best be remedied and avoided in the future.

In the area of legal affairs, special attention is given to the administration of cooperative research contracts with close support from the Finance Department. The majority of such contracts are established with industrial partners. In addition, matters involving jurisdiction aspects need to be prepared and, in many cases, legal opinions must be sought.

The Legal Department also deals with the commercialisation of all MDC patents. Contracts are negotiated and administered in close cooperation with both the patent lawyer and the technology transfer committee.

Head: Anja Ammar

Patents/Licences

The total number of German patents and patent applications from MDC in 2001 amounted to 116, compared with 105 in 2000. This included one patent which had awarded before MDC was established and which is still alive as drug certificate. In 2000 and 2001 the annual number of patent applications reached 22 each, compared with 20 in 1999. This is a sign of the stable innovative ability of the MDC researchers.

In the last 10 years 9 US patents for discoveries originating from MDC were granted. There are also 14 PCT (Patent Cooperation Treaty) patent applications now pending. In 2001 9 license agreements were conduded, among them 4 agreements with the company G.O.T. concerning liposomale encapsulated substances. The annual license income had a constant level in the last 2 years: 201 TDM (2000) and 196 TDM (2001).

The MDC had organized to check up all publication drafts to find possibly patentable objects. The Legal Protection Committee with Dr. Iduna Fichtner, Prof. Walter Birchmeier and Dr. Martin Lipp is responsible to suggest which applications should be made for foreign patents, in addition to the primary patent protection in Germany. This involves the inventors request to the MDC board and a subsequent checking procedure by our patent attorney, Dr. Fritz Baumbach.

Head: Dr. Fritz Baumbach

Technology Transfer

MDC scientists have increasingly become aware of aspects of Technology Transfer. This development is strongly supported by an evaluation of manuscripts for patentable results and methods before publication. Individual discussions initiated by a patent lawyer with group leaders at the MDC have led to an increasing awareness of commercial aspects of applied research.

Intensive contacts with patenting agencies have strengthened the process of commercialization of scientific results and resulted in an increasing number of licensing contracts.

As in former years, MDC scientists have founded spin-off companies. As a result of the BMBF supported project “Protein Structure Factory” two companies have been formed with participation of scientists from the MDC and FMP. One of them, PSF biotech AG, offers a broad technology platform for the development of novel pharmaceutic targets. The other one, Combinature Biopharm AG, uses NMR-supported technologies for drug investigation and development of lead compounds. The development, research and marketing of novel anticancer agents is the main expertise of NEMOD New Modalities Biomedical Development mbH.

Prof. Birchmeier is co-founder and Dr. von Kries scientific manager of the Semaia Pharmaceuticals GmbH dealing with the identification and development of novel tumor therapeutics.

MDC scientists were also involved in the foundation of the companies ReceptIcon and Motogena GmbH which are located outsite Berlin-Buch.

The MDC was actively involved in the establishment of a joint commercialization agency, Ascenion GmbH, which offers exclusive and comprehensive intellectual property asset management for the Life Sciences research institutes of the Hermann von Helmholtz-Association.

Head: Dr. Iduna Fichtner

Ascenion GmbH
Director: Dr. Christian A. Stein
Ingolstädter Landstraße 1
D 85764 Neuherberg /München
Tel.: (089) 3188 140
Fax: (089) 3188 1420
e-mail: stein@ascenion.de
www.ascenion.de

MDC-Neujahrsveranstaltung am 28. Januar 2000: (v. l.) Prof. Werner Franke (Deutsches Krebsforschungszentrum, Heidelberg), Wolf-Michael Catenhusen (MDC-Kuratoriumsvorsitzender und Parlamentarischer Staatssekretär im Bundesforschungsministerium), Christa Thoben (damalige Berliner Wissenschaftssenatorin) und Prof. Detlev Ganten (MDC-Stiftungsvorstand)
MDC`s New Year Reception on January 28, 2000: (from left): Prof. Werner Franke (German Cancer Research Center, Heidelberg), Wolf-Michael Catenhusen (chairman of MDC`s Board of Trustees and Parliamentary State Secretary from the Federal Ministry for Education and Research), Christa Thoben (former Senator for Science, Berlin) and Detlev Ganten (MD., Ph.D, MDC`s Scientific Director)
Copyright: MDC; Siegfried Endruweit

Press and Public Relations

Research at the MDC is conducted at the frontiers of biomedical science - in the field of molecular medicine. The MDC´s research activities need to be communicated to the general public in a way that can be readily understood. In addition, the cost of running the MDC must be justified to the German taxpayer. It is supported by public funds, like all other 15 institutions in the Helmholtz-Association of National Resarch Centers. The Press Office carries out a range of activities to serve this need which are outlined below.

In 2000 and 2001 the MDC Press Office organized more than 30 guided tours for almost 500 visitors - university students, high school students, international delegations, and the general public. These tours included lectures and visits to the laboratories of the MDC, the cooperating university affiliated clinics, the Robert Rössle Cancer Center and the Franz Volhard Clinic for Cardiovascular Diseases, as well as the Life Science Learning Laboratory at the Biomedical Research Park.

In 2000 and 2001 the MDC Press Office also continued the series of popular scientific lectures in the City Hall of Berlin Pankow, initiated in 1992, the MDC´s first year of existence, with 18 “Sunday Lectures” given by scientists from the MDC and other scientific institutions in Germany. It also presented the MDC`s research activities at various exhibitions e.g. in China (Deutsche Forschungsgemeinschaft, DFG), at the World Fair Expo 2000 in Hannover including two EXPO projects on the Berlin-Buch Campus, an exhibition of the Helmholtz-Association at the Deutsche Museum in Munich, the Biotechnology Fair “Bio 2001” in San Diego, USA, the Science Fair in Berlin and Warsaw (Poland) and various activities as part of the “Year of the Life Sciences” and “Wissenschaft im Dialog”, including the first “Long Night of the Sciences”.

A total of 97 news releases published in both German and English by the MDC Press Office in 2000 and 2001 formed the basis for many reports in the media. In addition, the MDC Press Office was also in overall charge of press relations for

Presse- und Öffentlichkeitsarbeit

Als Forschungsinstitut, das molekularbiologische Grundlagenforschung und klinische Forschung miteinander verknüpft, richtet sich das Augenmerk der Presse und der Öffentlichkeit bei der Biomedizin und der Genforschung in den vergangenen Jahren verstärkt auf das Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch. Das MDC wird wie alle weiteren 15 Einrichtungen der Helmholtz-Gemeinschaft Deutscher Forschungszentren (HGF) mit öffentlichen Geldern finanziert. Es ist deshalb bemüht, einer breiten Öffentlichkeit und damit dem Steuerzahler in leicht verständlicher Form zu vermitteln, für welche Forschungen es diese Mittel einsetzt. Die MDC-Pressestelle hat dazu folgende verschiedene Aktivitäten initiiert:

Im Jahre 2000 und 2001 betreute die MDC-Pressestelle in über 30 Führungen nahezu 500 Besucher – darunter Studenten, Schüler und internationale Delegationen. Die Pressestelle bereitete die Besuchsprogramme inhaltlich und organisatorisch vor und betreute mit den Wissenschaftlerinnen und Wissenschaftlern die Gäste. Die Führungen beinhalteten Vorträge, Laborbesuche im MDC, in den Kliniken sowie in Firmen des Biotechnologieparks. Eingeschlossen in diese Besuchsprogramme ist auch das Gläserne Labor der BBB Management GmbH.

Die MDC-Pressestelle führte im Berichtszeitraum die Serie populärwissenschaftlicher Sonntagsvorlesungen im Rathaus Berlin-Pankow fort, die das MDC 1992, dem Jahr seiner Gründung, initiiert hatte. Referenten sind Wissenschaftler des MDC, Kliniker der Robert-Rössle- und Franz-Volhard-Klinik sowie anderer wissenschaftlicher Einrichtungen im Bundesgebiet.

Weiter präsentierte sich das MDC in verschiedenen Ausstellungen im In- und Ausland, so in China bei einer Ausstellung der Deutschen Forschungsgemeinschaft (DFG), in Hannover mit der Helmholtz-Gemeinschaft auf der EXPO 2000 sowie mit zwei Expo-Projekten auf dem Campus Berlin-Buch. Es beteiligte sich des weiteren an der Helmholtz-Ausstellung „Lebendige Wissenschaft“ im Deutschen Museum in Mün-

the first joint Memorial of German Science for the Victims of the Nazi Regime, which was erected jointly by the Max Planck Gesellschaft, the Deutsche Forschungsgemeinschaft and the MDC. About 6 000 newspaper articles, with a circulation of well over 600 million copies, were published on aspects of research carried out at the MDC, the Robert Rössle and Franz Volhard Clinics, and the Biotechnology Park. In addition, 20 television and 30 radio productions, including the French/German TV channel Arte, the national TV channels ZDF and ARD as well as the Deutschlandfunk, were devoted to the research conducted at the MDC, its clinics and the Biotechnology Park.

In the time-span reported, three press conferences were planned, organized and run by the MDC Press Office. One of these press conferences, for example, was held at the "2nd International Symposium on Obesity and Hypertension" in Berlin-Buch in 2001.

In 2000 and 2001 the MDC Press Office prepared and organized more than 600 interviews for the media in Germany and abroad, including newspapers, magazines, television and broadcasting stations and scientific journals such as Nature, Science and The Lancet. In addition, the Press Office prepared many contributions about the MDC and its research for brochures such as the Helmholtz Association of German Research Centers. It also published four press reports summarizing the coverage of the MDC, its clinics, the Biotechnology Park, and related topics in the printed press as well as two issues of the MDC Report, an in-house magazine.

Head: Barbara Bachtler

chen. Zusammen mit der BBB Management GmbH nahm das MDC an der Bio 2001 in San Diego, USA teil. Mit dem Gläsernen Labor beteiligte es sich auf der Science Fair in Berlin und in Warschau (Polen) sowie an verschiedenen Aktivitäten im Rahmen des „Jahrs der Lebenswissenschaften" und von „Wissenschaft im Dialog" in Berlin. Dazu gehörte auch die erstmals durchgeführte „Lange Nacht der Wissenschaften".

Die MDC-Pressestelle recherchierte, verfasste und veröffentlichte im Berichtszeitraum 97 Pressemitteilungen in Deutsch und Englisch, die den Medien Anlass zu einer breiten Berichterstattung boten. Die MDC-Pressestelle war darüber hinaus federführend bei der Pressearbeit für das erste gemeinsame Mahnmal der Deutschen Wissenschaft für die Opfer des Nazi-Regimes, das die Max-Planck-Gesellschaft und die Deutsche Forschungsgemeinschaft mit dem MDC errichtet haben. In den Jahren 2000 und 2001 erschienen über 6.000 Zeitungsartikel, die einer Auflage von mehr als 600 Millionen entsprechen, in denen über die Forschungsarbeiten des MDC mit dem Campus Berlin-Buch berichtet wurde. Hinzu kamen mehr als 20 Fernseh- und 30 Hörfunkbeiträge, darunter auch Berichte in den Hauptnachrichtensendungen von ARD und ZDF sowie im Deutschlandfunk und im deutsch-französchen Kanal Arte.

Die MDC-Pressestelle führte im Berichtszeitraum drei Pressekonferenzen durch, die sie inhaltlich und organisatorisch vorbereitet hatte. Dazu zählte zum Beispiel eine Presseveranstaltung im Rahmen des „2. Internationalen Symposiums über Adipositas und Bluthochdruck" in Berlin-Buch im Jahre 2001.

Die MDC-Pressestelle vermittelte in den vergangenen zwei Jahren über 600 Interviews für Tageszeitungen, Magazine, Wochenzeitungen, Fernsehen, Hörfunk sowie für wissenschaftliche Magazine wie Nature, Science und The Lancet, die sie inhaltlich und organisatorisch vorbereitete. Weiter verfasste die Pressestelle mehrere Beiträge für verschiedene Broschüren, darunter auch der Helmholtz-Gemeinschaft. Weiter gab das MDC vier Pressespiegel sowie zwei Ausgaben des MDC-Reports, einer Zeitschrift des MDC für Mitarbeiterinnen und Mitarbeiter, heraus.

Leiterin: Barbara Bachtler

Administration

Personnel

The department is responsible for all matters relating to staff, wages, salaries, separation allowances, removal and travel expenses etc..

During the last ten years, MDC has not only managed to function efficently as a unified body, but stuff also work in a close harmony. During 1992, a total of 382 stuff were employed by MDC and, by December 2001 this figure had risen to 692, including those (207) paid by third-party funding. As before, most (89 percent) of the scientists' contracts are limited to a maximum of five years.

MDC is currently financing 17 graduate students studying for a PhD, who are not included in the list of employees. In addition, at MDC, there are 78 part-time, third-party financed young scientists.

Of the 294 scientists, 83 come from 32 different countries.

Head: Dr. Hans-Joachim Seehrich

Finances

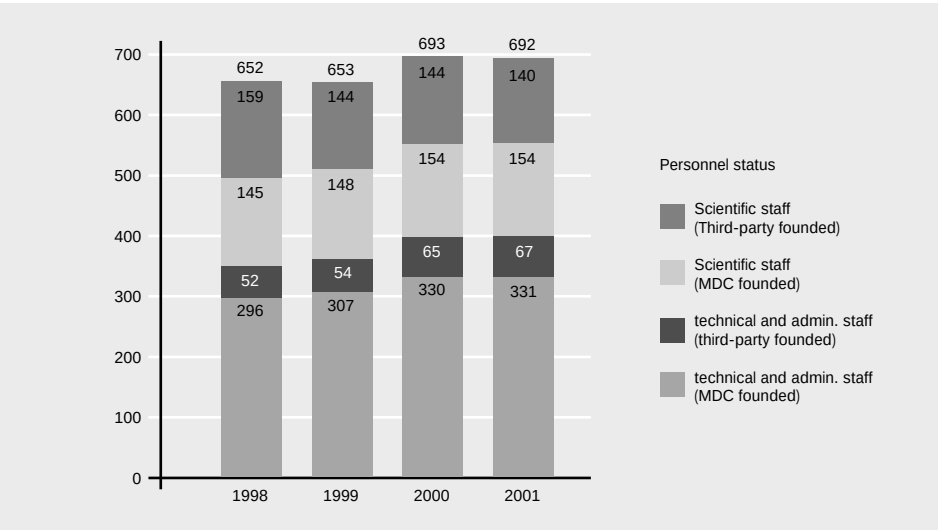
The Finance department concerns itself with all matters relating to the financial funding of the MDC, including accounting. The primary source (90%) of annual MDC funding comes from the Federal budget (Federal Ministry of Education and Research). The remaining 10 percent is provided by the State of Berlin (Senate Administration for Science, Research and Culture). Within the framework of its basic funding, MDC will receive 55 million Euro for the year 2002; approx. 15 million Euro (as of December 2001) will be made available in 2002 from third-party financial sources.

Increases have also taken place in the staff sector, due to general wage increases and, in particular, increases within Berlin. There has been a decline in recent spending on equipment after a period of heavy investment during the initial years of the MDC. Significant expenditure is necessary to maintain the MDC laboratories in their present functional state. Another positive development has been the attraction of third-party finance and, in 1996, the MDC was able to spend 9 million Euro of third-party income.

As mentioned above, approval for approx. 15 million Euro of extra-mural funding has already been received for the year 2002.

Head: Wolfgang Kühlewind

Personnel status. Distinctions according to financial sources.



Purchasing and Materials Management

The work of the Purchasing and Materials Management Department is focused on three main areas:

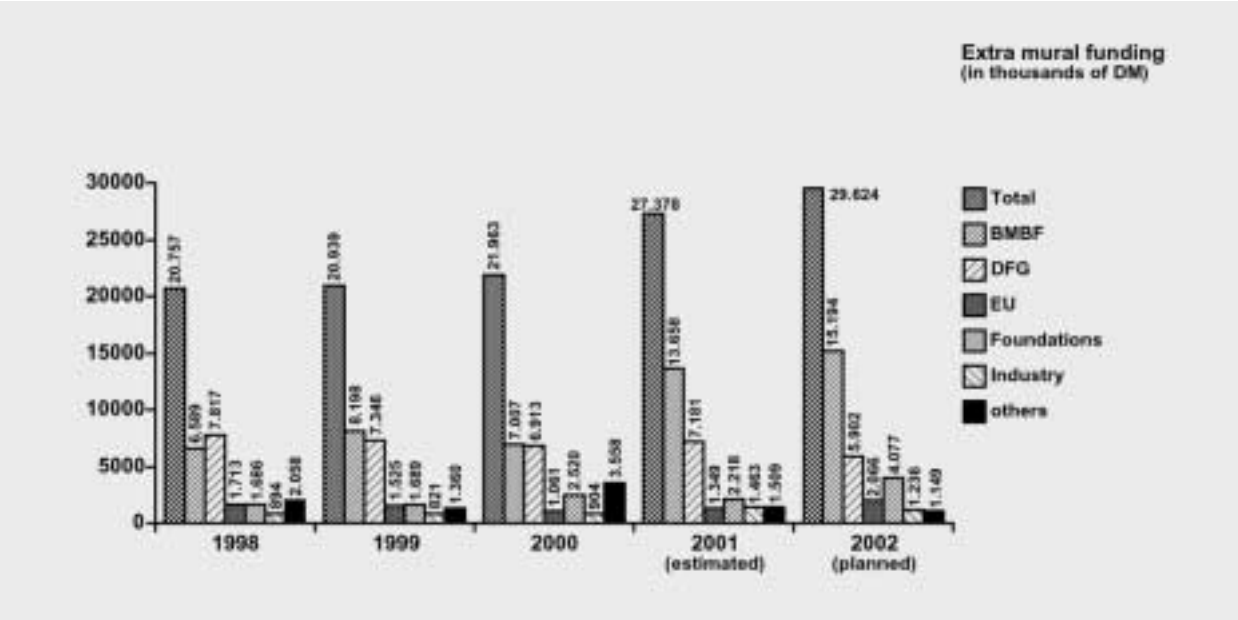
- rapid and efficient supply of quality laboratory materials, auxiliary and consumable items, and equipment at cost-effective rates
- step-by-step introduction of a decentralized ordering department, to implement an effective and transparent form of purchasing
- revision and compilation of new, up-to-date rules of procurement.

Over 16.300 orders are processed yearly. Compared with the MDC's early years, this is approximately the same number as last year. There were also 9,800 orders for chemicals in the year 2000.

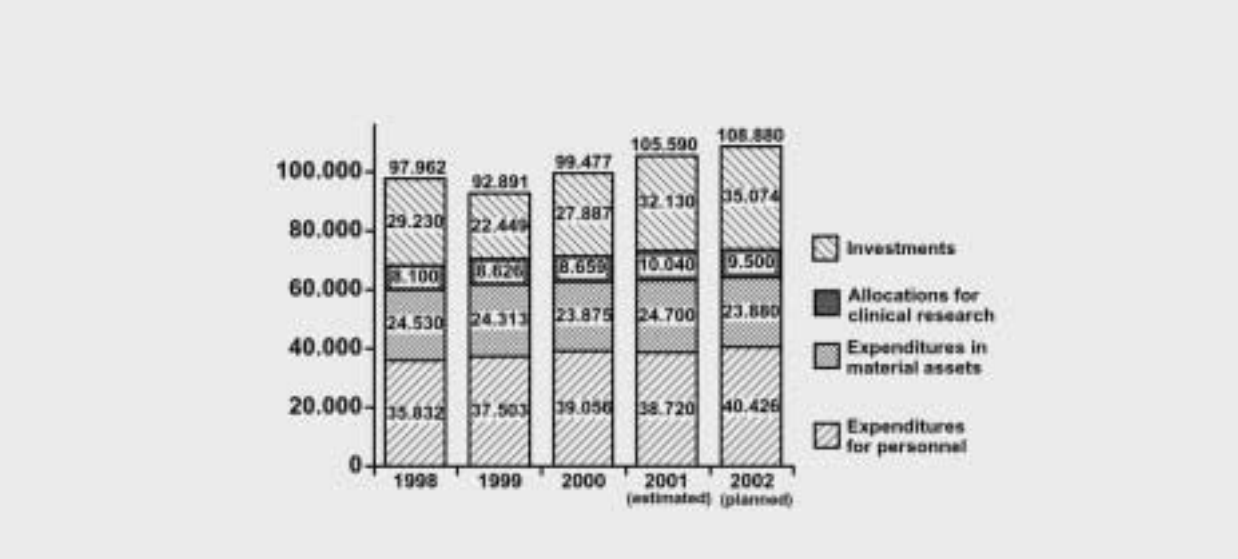
The step-by-step introduction of a decentralized ordering system will ease the workload of the department. After hooking up to the company's internal data processing network, employees will be able to log in and place their orders themselves. This will guarantee fast processing of purchasing. Linking the scientist' work places with the purchasing department is also an important requirement for the future transfer of data to appropriate laboratory and specialist suppliers, upon conclusion of general agreements and calling-forward contracts.

Head: Dr. Peter Konzer

MDC's extra mural funding (in thousands of DM)



Budget of the MDC (in thousands of DM)



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 center 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 43 000 media items and 250 print periodicals as well as different kinds of non-print materials. In addition to the print subscriptions, the library offers more than 900 electronic fulltext journals. Campus-wide provision of major local databases include Medline (starting from 1966), Current Contents Connect (5 Science Editions) as well as Web of Science (starting from 1980) and CD-ROM databases via an internal MDC-network with a range of scientific options.

The open area provides 26 reading desks and 5 computer workstations with internet access. The library operates with the local library computer system SISIS. The OPAC (Online Public Access Catalogue) lists all collections, starting from 1994, 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

Animal Facilities

Animal experiments make a major contribution to biomedical research, particularly in such complex areas as cardiovascular, cancer and neurological diseases. Animals experiments are especially concerned with the development of methods for improved diagnosis and treatment of human tumors and cardiovascular diseases. They are essential in establishing gene therapy strategies. With the recent development of powerful new technologies for manipulating genes, scientists worldwide have produced thousands of transgenic animals and knock-out models. Both approaches have become invaluable in studies of gene function in disease. In 1993 a transgenic unit was set up, which allows the production of transgenic rats and mice and mouse chimeras. Since then, more than 90 transgenic rat strains have been developed to model hypertension. In addition, mice were reconstructed by ES-cell injection and have been successfully bred since 1995. More than 300 strains of knock out mice are now available as experimental models in cancer research, cardiovascular and neurological diseases. The animal facility of the MDC posesses within the research institutes of Berlin the most genetically engeneered rat strains and mice stocks.

The four animal houses support transgenic and animal experimentation at the MDC. Mice, rats and rabbits are bred. Located on a 1260 square-meter area, the facilities include animal rooms (636 m2), surgery rooms, storage rooms and cage washing facilities. The animals are bred in a disease-free environment. Nevertheless, the number of available animal rooms and surgical facilities in and around the MDC does not meets present requirements.

The rapidly growing number of genetically engeneered models of severe human illness means, that further animal facilities for breeding and experimentation are needed. The MDC therefore builds up a new central animal house. This new facility possesses substancial more capacity for animals (29 animal rooms on a 1015 square- meter area) and laboratories (19 labs on 665 square meters). This animal house will be opened by the year 2003.

Head: Dr. Karin Jacobi

Campus Net Management

The wide area computer network at the Berlin-Buch Research Campus is managed by BBB Management GmbH. In particular, its Campus-IT team is responsible for the operation of the high speed connection with the Internet via the recently opened G-Win (scientific network, Internet-II) connection of the DFN-Verein. The campus network is supplied with all necessary internet services, such as as E-Mail, World Wide Web, FTP, and a Phone Directory. A dedicated Web site to provide information about the Campus in general is under development and will be launched at the beginning of 2002. Further services for the Campus, like video-conferencing and IP-traffic billing/controlling, have been established during the last three years.

Head: Hans Mitulla

Data and Image Processing

The group manages the central computer facilities of the MDC (Remote Access-, File, and Backup-servers) and is responsible for the client/server operation of the MDC's Administration and Executive Board, and the system- and user-support of the SAP-R/3- administration system.

Bei sommerlichen Temperaturen auf der Terrasse des Café Max auf dem Campus Berlin-Buch
Summertime on the Berlin-Buch Campus, sitting on the terrace of the Café Max

The group routinely supports users if there are any hardware and software problems, connects PC, Macintosh and local nets with the MDC net, and installs client software for different computer platforms for the use of both the central computer facilities of the MDC and the campus-wide computer facilities of the BBB GmbH.
New tools for software-distribution and remote control are now undergoing testing.

In addition, the group organises standard software courses in our computer laboratory.

Our computer laboratories for image processing provide support for the research groups if there are any scientific problems involving image-processing, data-analysis and -visualisation as well as in the presentation of scientific results (graphics, slides, posters). The latest technology, such as digital photography, video-digitalisation and -processing has been installed.

The group is now engaged in introducing new security solutions for the MDC computer network.

Head: Bernd Lemke

Technical Affairs

During the report period 2000/2001 the Technical Department completed the fine-tuning of the air-conditioning equipment that had been installed after completion of all the renovation work.

The Technical Department also completed the construction work in Building 31.1 (Ground Floor and Floors 1 and 5, Building 31.3 (Cryo-Storage) as well as Building 64 and handed them over to their future occupants.

Conversion work was successfully completed in the MDC "Connection Techniques" building involving the "Photon Surface" (Windows). This provided the maximum amount of user comfort and transparency for all technical operating equipment. This will allow different users to "dial into" installations via the data network (e.g. equipment in the animal house, monitoring the operation of deep freezes etc) and to control operating conditions and, if required, make appropriate changes using the control system.

Air-conditioning is being installed in the new X-ray laboratories in the basement of the MDH so that the monitoring facilities in these areas will be operational by the end of the year.

The Technical Department has been working flat out to keep to its schedule for linking the new Congress Center with existing MDC services (telephone, access controls, media facilities etc.).

During the period covered by this report, a start has been made in optimizing all the equipment that has been acquired, as well as checking that the technical documentation is complete and ensuring that all revisions have been incorporated in the documentation; revision of all acquired CAD data support systems to ensure uniformity of operating conditions; development of key technical operating data for all equipment with the aim of allowing the optimal allocation of resources for servicing and maintenance to companies and service providers as well as improving the overall management with only a few members of staff from the Technical Department.

Head: Harry Schenk

Meetings, Workshops and Symposia

Neue Wege in der Diagnostik von Herzerkrankungen: Interessierte Besucher lassen sich während der „Langen Nacht der Wissenschaften“ am 15. September 2001 in der Franz-Volhard-Klinik der Charité / Helios Kliniken GmbH die Magnetresonanz-Tomographie erklären.
New methods for diagnosing heart diseases – Interested visitors are told about magnetic resonance tomography during the “Long Night of the Sciences” on September 15, 2001, at the Franz Volhard Clinic of the Charité / Helios Kliniken GmbH.
Copyright: BBB GmbH; Thomas Oberländer / Helios-Kliniken

Impressionen von der ersten „Langen Nacht der Wissenschaften“ auf dem Campus Berlin-Buch am 15. September 2001
Impressions of the first “Long Night of the Sciences” on the Berlin-Buch Campus, September 15, 2001
Copyright: BBB GmbH; Thomas Oberländer / Helios-Kliniken

The following events organized under the auspices of the MDC and its clinical partners took place in 2000 and 2001

2000

4th Workshop Interventionelle Kardiologie, Franz Volhard Clinic for Cardiovascular Diseases
(January 28, 2000)

BIO 2000 International Meeting & Exhibition
(March 26-30, 2000, Boston, MA, Hynes Convention Center)

Coping with Sarcoma –Functional, Psychological and Social Dimensions
Robert Rössle Cancer Center, Charité and Freie Universität Berlin
(April 13, 2000)

MDC-Neuro-Meeting
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
(May 4-5, 2000)

6th MDC Graduate Students’ Symposium
(May 17, 2000)

“Neue Therapiestrategien in der Hämatologie: Was ist gesichert?” im Rahmen des 49. Deutschen Ärztekongresses Berlin
(May 30, 2000)

Experten Meeting “Medical Genomics”
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
(June 5 and June 23, 2000)

Symposium Brain Tumors
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
(June 15-16, 2000)

6th Bucher Symposium “Science Meets Clinic”
Franz Volhard Clinic for Cardiovascular Diseases, Campus Berlin Buch
(July 1, 2000)

29th European Muscle Conference
Max Delbrück Center for Molecular Medicine (MDC) Berlin Buch and Charité, Humboldt University, Berlin
(September 8-13, 2000)

Symposium zum 100. Geburtstag von Nikolai Wladimirovich Timoféeff-Ressovsky “Genetik in Russland und Deutschland”
Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch
(September 21-22, 2000)

Berlin Lectures on Molecular Medicine with Joan A. Steitz, Sterling Professor of Molecular Biophysics and Biochemistry, Howard Hughes Medical Institute, Research Laboratories, Yale University New Haven, Connecticut, USA
(Charité Medical Faculty of the Humboldt University of Berlin)
(October 26, 2000)

HGF-Workshop "Strategic Consideration of Medical Imaging and Robotics"
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
(November 13-15, 2000)

4th Congress of Molecular Medicine (CMM) and VIIth Franz-Volhard-Symposium,
1st Cardiac Cachexia Symposium Conference
(December 1-2, 2000)

Bucher Symposium "Neuroscience"
(December 18–19, 2000)

2001

MDC Symposium "Molecular Cell Biology and Gene Therapy"
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
(February 13, 2001)

3. Zellbiologie Symposium
Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch
(March 21-25, 2001)

10th International Symposium on SHR and Molecular Medicine
Rat Genetics, Genomics and Model Systems for Human Diseases
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
(May 2-4, 2001)

Brain Tumors 2001, A Multidisciplinary Overview
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
(May 10-11, 2001)

7th Graduate Students' Symposium
Max Delbrück Center for Molecular Medicine (MDC) and Forschungsinstitut für Molekulare Pharmakologie (FMP)
(May 14, 2001)

IV. Onkologische Fachtagung zum Thema: Symptome in der Onkologie
Robert Rössle Cancer Center/Franz Volhard Clinic for Cardiovascular Diseases/Universitäts-Klinikum Charité der Medizinischen Fakultät der Humboldt-Universität zu Berlin, Campus Berlin-Buch
(May 30-June 1, 2001)

BIO 2001 International Convention and Exhibition
(June 24-28, 2001, San Diego Convention Center, USA)

2nd International Symposium on Obesity and Hypertension Genetics and Molecular Mechanisms
Max Delbrück Center for Molecular Medicine (MDC) Berlin Buch
(October 25-27, 2001)

Cell Migration in Development and Disease
Max Delbrück Center for Molecular Medicine (MDC) Berlin Buch
(November 28 - December 1, 2001)

Berlin Lectures on Molecular Medicine with Eric S. Lander, Whitehead Institute, Cambridge, USA,
Max Delbrück Communications Center (MDC.C)
(December 5, 2001)

Scientific Exhibitions

Unsichtbar – Sichtbar – Durchschaut:
Das Mikroskop als Werkzeug des Lebenswissenschaftlers
(Invisible-Visible-Transparent: The Microscope as a Tool for the Life Scientist)
September 10-16, 2001
Deutsches Technikmuseum Berlin

Schwarzer Tod und Amikäfer:
Eine Wanderausstellung zur Geschichte der Biowaffen
(Black Death and Ami-Beetle: A Traveling Exhibition to the History of Bioarms)
MDC and "Wissenschaft im Dialog"
2000-2002
Castle Sachsenburg, Berlin, Dresden

Eine Reihe von rund 200 Jahre alten Mikroskopen aus der vom MDC organisierten Ausstellung „Unsichtbar – Sichtbar – Durchschaut", die anläßlich des Wissenschaftssommers 2001 im Technik-Museum Berlin zu sehen war. Im Hintergrund eine Linsenschleifmaschine (l.) und eine Drehbank (re.), mit denen solche Forschungsinstrumente hergestellt wurden.
A series of microscopes about 200 years old from the exhibition "Invisible - Visible - Transparent", which the MDC had organized and which was presented as part of the "Scientific Summer 2001" in the Technical Museum, Berlin. In the foreground is a lens polishing machine (left) and a lathe (right), which were used to construct these research tools.
Copyright: MDC; Peter Martin

Awards

2000

MDC and Forschungszentrum Karlsruhe
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Head of the Scientific Committee of the MDC 1992-2000

2000
Joan A. Steitz
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2001
Eric S. Lander
Whitehead Institute, Massachusetts Institute of Technology, Cambridge/USA

Auf der MDC-Neujahrsveranstaltung am 19. Januar 2001: Hans-Jürgen Delbrück von der Delbrück'schen Familienstiftung (rechts) im Gespräch mit dem Physikstudenten Ermin Malic, Gewinner des von der Stiftung und dem MDC vergebenen „Abiturienten-stipendiums".
At the MDC's New Year Reception on January 19, 2001: Hans-Jürgen Delbrück from the "Delbrück'sche Familienstiftung" (right) chats with physics student Ermin Malic, winner of the "Abiturientenstipendium", which is awarded by the Delbrück-Foundation and the MDC.

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