

Research Report 2000

Covers the Period 1998/99

Content

Foreword		4
Introduction		7
Genetics, Bioinformatics and Structural Biology		18
	• <i>Molecular Biology and Genetics of Cardiovascular Diseases</i>	Detlev Ganten 20
	• <i>Molecular Biology of Peptide Hormones</i>	Michael Bader 22
	• <i>Genome Research, Gene Variation, and Complex Disease</i>	Margret Hoehe 24
	• <i>Etiology and Pathogenesis of Hypertension and Vascular Disease</i>	Friedrich C. Luft 26
	• <i>Gene Mapping and Identification in Monogenic and Complex Diseases</i>	André Reis 28
	• <i>Tumor Genetics</i>	Siegfried Scherneck 30
	• <i>Clinical and Molecular Genetics of Cardiovascular Diseases</i>	Herbert Schuster 33
	• <i>Mouse Genetics</i>	Carmen Birchmeier 36
	• <i>Developmental Genetics</i>	Andreas Schedl 38
	• <i>Lipids and Experimental Gene Therapy</i>	Thomas E. Willnow 40
	• <i>Bioinformatics</i>	Jens Reich, Peer Bork 42
	• <i>Interactions of Biopolymers in Solution</i>	Joachim Behlke 44
	• <i>Folding and Misfolding of Proteins</i>	Gregor Damaschun 46
	• <i>Protein Folding and Misfolding</i>	Heinz Fabian 48
	• <i>Structural Studies of Proteins and Nucleic Acids by X-ray Crystallography</i>	Udo Heinemann 50
	• <i>Role of Protein Dynamics in Enzyme Function</i>	Christiane Jung 53
	• <i>Modeling Nucleic Acid Structure and Recognition by Proteins</i>	Heinz Sklenar 55
	• <i>Conformation, Stability and Interaction of Biological Macromolecules</i>	Heinz Welfle 57
	• <i>Protein Structure Analysis and Proteome Research</i>	Brigitte Wittmann-Liebold 59
Cell Growth and Differentiation		62
	• <i>Growth Control and Gene Regulation in the Hematopoietic System</i>	Achim Leutz 64
	• <i>Regulation of Transcription in Mammalian Cells</i>	Claus Scheidereit 67
	• <i>Differentiation and Growth Control in Lymphocyte Development and Function</i>	Martin Lipp 69
	• <i>Mechanisms Controlling the Initiation of DNA Replication</i>	Manfred Gossen 72
	• <i>Cell Cycle Regulation</i>	Hans-Dieter Royer 73

• <i>Epithelial Differentiation, Invasion, and Metastasis</i>	Walter Birchmeier	75
• <i>Cell Adhesion and Signal Transduction</i>	Jürgen Behrens	78
• <i>Glycoconjugates</i>	Uwe Karsten	80
• <i>Intracellular Signaling in the Myocardium</i>	Ernst-Georg Krause	82
• <i>Molecular Muscle Physiology</i>	Ingo L. Morano	84
• <i>Cell Biology of Cardiovascular Diseases</i>	Heinrich Leonhardt, M. Cristina Cardoso	86
• <i>Surgical Oncology</i>	Peter M. Schlag	89
• <i>Ubiquitin System and Endoplasmic Reticulum</i>	Thomas Sommer	91
• <i>P450 Cytochromes and the Endoplasmic Reticulum</i>	Wolf-Hagen Schunck	93
• <i>Vascular Biology</i>	Hermann Haller	95
• <i>Functions of Dynamin II and PKC in Post-Golgi Vesicle Formation</i>	Peter Westermann	98
• <i>Electron Microscopy</i>		99
Molecular Therapy		102
• <i>Hematology, Oncology and Tumor Immunology</i>	Bernd Dörken	103
• <i>Molecular Immunotherapy</i>	Antonio Pezzutto	106
• <i>Experimental Pharmacology</i>	Iduna Fichtner	108
• <i>Drug Targeting</i>	Regina Reszka	110
• <i>Molecular Basis of Congestive Heart Failure</i>	Rainer Dietz	113
• <i>Immunology of Cardiovascular Diseases</i>	Gerd Wallukat	114
• <i>Molecular Immunology and Gene Therapy</i>	Thomas Blankenstein	116
• <i>Cellular Immunology</i>	Kirsten Falk, Olaf Röttschke	118
• <i>Molecular and Cell Biology of Hematopoietic Cells</i>	Martin Zenke	119
• <i>Phospholipids</i>	Dietrich Arndt	121
• <i>RNA Chemistry</i>	Eckart Matthes	122
• <i>Regulation and Deregulation of Cell Proliferation and Gene Therapy</i>	Research Group of the Humboldt University of Berlin at the MDC	123
• <i>Evolution, Regulation and Genetic Applications of Transposable Elements in Vertebrates</i>	Zoltán Ivics	125
Molecular and Developmental Neurosciences		128
• <i>Cellular Neurosciences</i>	Helmut Kettenmann	129
• <i>Growth Factor and Regeneration</i>	Gary R. Lewin	131
• <i>Synapse Formation and Function</i>	Frank W. Pfrieger	133
• <i>Developmental Neurobiology</i>	Fritz G. Rathjen	135
Structure and Organization		140
Organs of the Foundation		140
Supporting Divisions		143
Press and Public Relations		145
Administration		146
Central Facilities		148
Meetings, Workshops and Symposia		150
Awards		151
Addresses of Scientific Journals at the Berlin-Buch Campus		152
Index		153
Structure of the MDC		159

Foreword

“Molecular Medicine”: Progress by Interdisciplinary Research

At the beginning of the 20th century, physics was the dominant and fast-moving science. This has changed towards the end of the 20th century, when important progress was made in the biological and life sciences. During the last decades, the molecular basis of life was elucidated in its fundamental aspects. The basic mechanisms that make and maintain a living cell, for instance energy metabolism, replication of DNA, RNA and protein synthesis, are understood. The information can now be found in textbooks on biology or medicine in a condensed form. The final goal of modern life sciences, the understanding of a complex organism in molecular terms, is in reach. This implies that the molecular causes of human diseases can and will be elucidated.

Such knowledge must be applied to the maintenance of health, the diagnosis and treatment of human disease. I see our mission at the MDC in the establishment of a life science that improves the human condition. To fulfill the promises and expectations that arise from this, modern medicine must take advantage of many different disciplines and their methods. In addition to conventional anatomy, physiology, biochemistry, genetics, microbiology, pathology, and the clinical disciplines, new research areas such as genomics, proteomics, bioinformatics, and even “phenomics” have emerged. Other areas such as physics, informatics, material sciences, nanotechnologies etc. become increasingly important. Today disciplines are not as clearly defined as in the past: The application of molecular biology, genetics, and genomics to classical disciplines has blurred their traditional borders.

“Molecular Medicine” requires an interdisciplinary approach: on the one side the understanding of physiological and pathological processes on basic levels and, on the other side, the application of the knowledge to clinical challenges. Classical discipline-oriented research and educational institutions do not favor interdisciplinary interactions. Progress, however, can only be made through intelligent cooperations. Creative scientists have always looked beyond their own disciplines. We are coming to a point where not only individuals, but also entire research organizations and institutions need to think along such interdisciplinary lines. In my view, an interdisciplinary life science is the science of the future, a “living science” in the genuine sense of the word. When applied to the human condition, it is the essence of Molecular Medicine. At the Max Delbrück Center for Molecular Medicine, the “MDC”, we foster such cooperations between basic research and clinical disciplines.

Interdisciplinary research is pursued by the MDC, but also by all the other 15 members of the Helmholtz Association of National Research Centres. One of the aims of these centers is the establishment of programs that address complex problems by interdisciplinary, cross-country cooperation. At the MDC, this aim is addressed by assembling biomedical and clinical disciplines at one single center, and by fostering their cooperation. I am convinced that therein lies the future of medicine.

Let me look back in history and consider the beginnings of Molecular Medicine on a very selective bias, focusing on the example of Max Delbrück and his impact on interdisciplinary research in medicine.

The Rockefeller Foundation, that funded among others Max Delbrück, had a pivotal impact on medicine and the biological sciences at the beginning of the 20th century. An important medical textbook, the “Principles and Practice of Medicine” (Appleton and Co. New York, 1893) written by William Osler, provided the impetus for the establishment of the Rockefeller Foundation. It was the first medical textbook clearly describing diseases in a manner understandable to the layman. Osler’s book was very honest on the subject of therapy, which was basically non-existent. After he read this book, Frederick T. Gates, a non-physician, convinced the wealthy John D. Rockefeller to create this philanthropic foundation. The researchers, which were funded in Rockefeller’s program, included scientists from many different fields, for instance physics and chemistry. Warren Weaver, the director of natural sciences division of the Rockefeller Foundation, named the broad, well-funded program in 1938 with the new but now familiar term “Molecular Biology”. Weaver aimed to support “the application of theoretical and experimental procedures to the study of the organization and reactions of living matter”. This was the first major interdisciplinary biomedical program. Those at the Rockefeller Foundation understood better than anyone else that too many scientific efforts were conducted in isolation, but needed coordination. Born out of these ideas was the “Science of Man” program, a great success.

Max Delbrück from Berlin was one of many researchers that contributed and benefited from this program. He was at the center of a well-funded and intellectually fertile group, the founders of today’s molecular biology. His career provides a lesson for the advantages of interdisciplinary research. Max Delbrück was educated as a physicist. Another physicist, Niels Bohr, challenged him to start a revolution in biology similar to the one that was occurring already in physics.

The quantum theory, that provided a new basis for the understanding of matter, was put forward by Niels Bohr and others who studied the interaction between light and matter. Bohr speculated that a similar revolution could occur in biological sciences by applying quantum mechanics to living organisms. In Berlin, Max Delbrück came to know an equally young Russian geneticist who worked in Berlin-Buch, Nikolai Wladimirovich Timoféeff-Ressovsky. The two collaborated and developed ideas then unheard of. As an experimental system, they used *Drosophila* flies, which were irradiated to increase mutation rates. Their results gave the term “gene locus” a material basis and they concluded that genes were physical entities of a defined size, an assembly of atoms, “Atomverband”, as Max Delbrück called it. This was a major conceptual breakthrough published in the famous paper “On the Nature of Gene Mutation and Gene Structure” in 1935.

At this time, genetics was thriving as a discipline in its own right. Thomas Hunt Morgan was mapping genes in *Drosophila* by the analysis of their allelic association. Barbara McClintock was watching color changes in corn plants caused by “jumping genes”. When Max Delbrück received his Rockefeller

Foundation fellowship to study in the United States, he visited several laboratories and decided to establish himself at the California Institute of Technology in Pasadena. There, a lone maverick, Emory Ellis, was studying bacterial parasites called “phages”. Phages turned out to be the tool with which physicists like Max Delbrück revolutionized biology.

Max Delbrück’s ideas and his vision of the atomic constitution of genes stimulated the Nobel Prize-winning Austrian physicist Erwin Schrödinger, who left Berlin for Dublin in 1933, to write a monograph entitled “What is Life?” Other physicists, George Gamow, Leo Szilard, and later, Francis Crick, also started to apply their knowledge, soon making important contributions to biology. According to Schrödinger, the main trick of life rested in the capability to produce order from order, while order tends to decay towards disorder in inanimate matter, according to the law of entropy. He concluded that biologists had to know more about the gene’s structure to understand the secrets of living organisms. Until today, the elucidation of the structure and function of genes and proteins is a central and dynamic field of research.

That the chemical nature of genes is deoxyribonucleic acid (DNA) was

indicated by the experiments of O. T. Avery. Two persons established the physical structure of genes, i.e. of DNA, which immediately implied how genetic information can be propagated from one generation to the next. They were the young ornithologist James Watson and Francis Crick, a physicist turned molecular biologist. Watson and Crick provide another example that it often takes several disciplines and different lines of thought from separate individuals for a great discovery. They built upon important concepts about the nature of the chemical bond from Linus Pauling, and were aided by the X-ray defraction studies of Rosalind Franklin and Maurice Wilkins. Just like Delbrück and Schrödinger, Watson and Crick concentrated on a problem, and not on a traditional discipline.

The advent of molecular biology, which came about by this fruitful interaction between physicists and biologists, has lead within less than 50 years to the understanding of many fundamental biological processes. The effects of this research were extraordinarily profound, and stimulated many scientists. Molecular biology, cell biology and genome research are still strongly influenced by these historical developments. We now know in principle how a complete organism, such as a worm or fly, can emerge from an egg, and that many of these mechanisms are employed even in higher animals and man. We have learned how the cell controls growth, and, in parallel, what are the genetic causes of cancer. This knowledge can hopefully be applied to the treatment of this disease in the

Figure 1: Max Delbrück (1906 Berlin – 1981 Pasadena/USA) in his office at the California Institute of Technology in 1970, shortly after having been awarded the Nobel Prize for Physiology and Medicine in 1969. Collaborating closely with Nikolai Wladimirovich Timoféeff-Ressovsky and Karl G. Zimmer during his stay in Berlin between 1932 and 1937, he is considered one of the pioneers in the field of modern genetics and molecular biology, renowned for his work on bacterial viruses (phages).



Figure 2: The famous publication “On the Nature of Gene Mutation and Gene Structure” is considered a milestone in the history of modern genetics. It was published by the Berlin scientists Nikolai Wladimirovich Timoféeff-Ressovsky, Max Delbrück, and Karl G. Zimmer in 1935.

future. Glimpses of the molecular causes of other important human diseases, for instance cardiovascular disorders, are emerging. What is still lacking is a complete understanding of complex organism in molecular terms.

The complete genome sequences of various viruses, numerous bacteria, yeast, and of the first multicellular organism, *C. elegans*, have dramatically demonstrated what lies ahead of us. Already, the first complete sequence of a human chromosome is published; rough drafts of the sequence of all chromosomes will be available between 2001 and 2003. Quickly thereafter, the complete sequence of the human genome will be available to anyone with an Internet access. This information will create a completely new biology and medicine, provided we are able to make practical use of it. It can lead to new diagnostic and therapeutic avenues, vaccines, drugs, procedures, and genetic tests. This will also bring along thorny new questions about ethics, fairness, and privacy. The Molecular Medicine and the life science have therefore to be provided with responsibility, an open and public dialogue and a human touch. Subject matter and the research organization must respond to this challenge and must be in harmony.

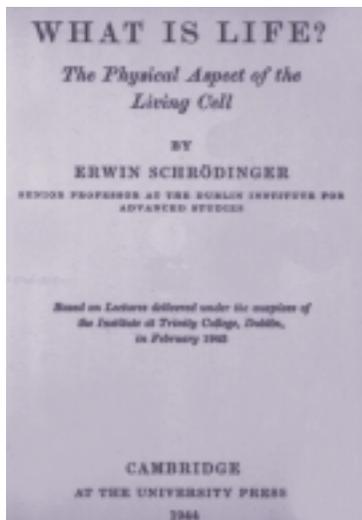
Figure 3: Nobel Laureate Erwin Schrödinger (1887 Vienna – 1961 Vienna), renowned for his studies on quantum mechanics and inspired by Max Delbrück's model of the genes as "Atomverband", successor of Max Planck at the University of Berlin from 1927 to 1933.



The future knowledge can only be successfully employed in medicine if we achieve such a harmony.

We can consider the Human Genome Project as today's "Science of Man" program. Weaver's "mathematical biology" anticipated the emerging theoretical biology and bioinformatics, in which the computer is as important as the experiment. The "Science of Man" program was singular in its success because it viewed the science of man as interdisciplinary. To use the information generated by the Human Genome Project will again require an interdisciplinary effort. I firmly believe that integrating genomic, genetic and medical research will lead us to a new science of man that we call Molecular Medicine. Molecular Medicine has great potential and also great responsibilities for bringing the disciplines together. We are convinced and dedicated to the notion that this goal can be achieved. Medicine has always benefited from discoveries made in physics (X-rays, isotopes), chemistry (reagents and pharmaceuticals), and engineering (biomaterials and instrumentation). Now new bridges must be constructed to link physics, biology, medicine, and other disciplines even closer.

Figure 4: Erwin Schrödinger's book "What is life", published in 1944, addresses the question, whether life can be explained by the laws of physics. Schrödinger worked on a model which described genes as "aperiodic crystals", referring to the famous work "On the Nature of Gene Mutation and Gene Structure" by Nikolai Wladimirovich Timoféeff-Ressovsky, who worked in Berlin-Buch, Max Delbrück, and Karl G. Zimmer.



At the MDC, we are determined to create an interdisciplinary research environment. These efforts are timely and well conceived. I am convinced that they will be of advantage for all and a disappointment for no one. We are trying our best at the MDC and we are grateful for your support.

Detlev Ganten

Figure 5: Nikolai Wladimirovich Timoféeff-Ressovsky (1900 Province Kaluga – 1981 Obninsk/Russia). He was one of the pioneers in the field of modern genetics and molecular biology, working in Berlin-Buch from 1925 to 1945.



Introduction

Clinical Research

The collaboration between the MDC and the two university clinics Franz Volhard Clinic for Cardiovascular Diseases and Robert Rössle Cancer Clinic (both Charité Medical School of the Humboldt University of Berlin) Berlin-Buch Campus, has developed in an exceedingly satisfactory manner in recent years. Annually, the MDC makes available about 14 million DM from its budget for joint research projects. The following procedures were set up very soon after the establishment of the MDC to allocate MDC research funds to clinics:

1. The four senior professors and physicians of the Franz Volhard Clinic and the Robert Rössle Clinic are simultaneously in charge of a research group at the MDC, provided with staff, materials and equipment, investment and laboratory space to a level comparable with that of the MDC research groups. 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. In recent years, two research groups have also been set up consisting of clinicians working part-time as guest researchers at the MDC. This ensures that there is an unhindered institutional link between clinical and basic research.
2. The improvement in the research infrastructure, as well as the setting up of Clinical Research Units (CRU), form the basis of collaborative projects under which particular diagnostic and therapeutic procedures can be carried out on patients and

volunteers. This provides a sound basis for clinical research. These Clinical Research Units 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 the 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 Franz Volhard Clinic as well as setting up a tumor bank at the Robert Rössle Clinic. 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.

Another important activity is the setting up of a GMP (Good Manufacturing Practice) laboratory at the MDC that will operate in collaboration with Schering AG to produce drugs to be used in gene therapy.

3. All the research funding which the MDC provides for Clinical Collaborative Projects (Klinische Kooperationsprojekte, KKP) is monitored by internal and external experts.

The financing of clinical research from MDC funds is based exclusively on research projects. These projects obtain financial contributions for research-associated additional expenditure

provided that it involves collaborative projects between the MDC and the clinics. These KKP 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. Currently, 13 such projects are underway. A further 6 projects are due to start shortly. In general, they run for 2 – 3 years. The maximum period of funding is 5 years.

This allows an important goal to be achieved, namely 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.

4. Clinical Training Program (Klinisches Ausbildungsprogramm, KAP) for young doctors/internal. The fundamental idea behind this clinical training program 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 a foundation for their further scientific development. At present 16 young doctors are taking part in this program.

These four sponsorship programs form a substantive unit and have been set up by means of agreed procedures and evaluated. This guarantees that outstanding clinical research projects can be supported for specific periods from MDC resources.

Gene Therapy Program

One of the most important scientific and applied areas of cooperation between the MDC and the clinics involves the development of strategies and procedures for gene therapy. As far as this topic is concerned, 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 German Federal Ministry for Education and Research (Bundesministerium für Bildung und Forschung, BMBF) for a period of 2 x 4 years.

The Good Manufacturing Practice (GMP) laboratory includes four laboratories for vector production and two for analysis and cell culture. The GMP laboratory is operated in conjunction with Schering AG and is also available for use in collaborative projects with other partners e.g. biotechnology companies located on the Campus.

Patient-oriented Research: Highlights in the Clinics

The Franz Volhard Clinic houses two departments, the Department of Cardiology and the Department of Nephrology/Hypertension. Rainer Dietz and Friedrich C. Luft are the respective heads. The Robert Rössle Clinic encompasses two departments, the Department of Hematology/Oncology and the Department of Oncological Surgery. Bernd Dörken and Peter M. Schlag are the respective heads. The four chiefs are fully aware that the goal of the unique cooperation with the MDC is to help clinicians formulate better hypotheses to pose at the bedside, as well as to expedite the transfer of basic knowledge into clinical practice.

In the Franz Volhard Clinic two cardiologists, Ludwig Thierfelder and Matthias Friedrich, have teamed up to investigate arrhythmogenic right ventricular cardiomyopathy (ARVC). Usually, the cause for the responsible cardiac arrhythmia is never identified. ARVC, which is inherited both as a dominant and recessive trait, is responsible for sudden death in young people. The condition may be more common than believed because of new diagnostic tools that can be applied before symptoms (fatal ventricular arrhythmias) develop. With the help of genetic field working, entire families can be studied to identify asymptomatic individuals. Such persons can be given medication prophylactically or treated with implantable defibrillators. In the Franz Volhard Clinic, Jens Jordan and other clinicians are studying mechanisms of high and low blood pressure. The group is studying a rare form of monogenic hypertension with brachydactyly.

Affected persons also feature as an additional phenotype, an aberrant looping posterior-inferior cerebellar artery, that impinges on the ventrolateral medulla. This so-called PICA loop could interfere with baroreceptor reflex signaling. Similar PICA loops are commonly observed in patients with primary essential hypertension, raising the hypothesis that a new, hitherto unrecognized form of secondary hypertension exists. The group has also identified a defect in a norepinephrine transporter that interferes with sympathetic function.

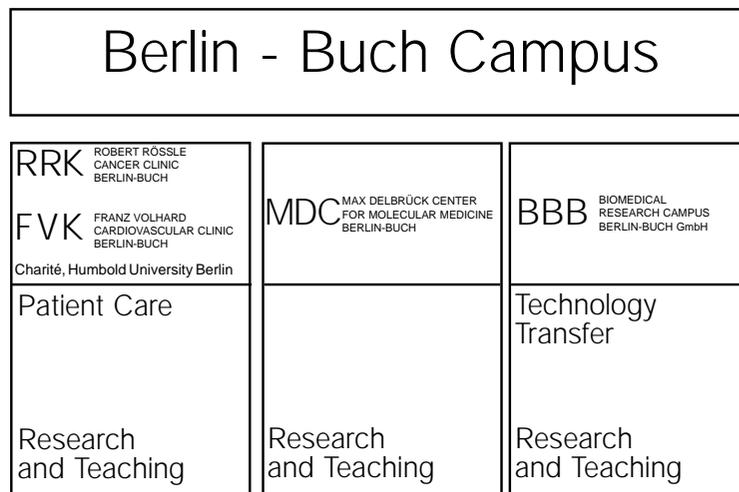
In the Robert Rössle Clinic, clinical scientists are working to offer patients with renal cell carcinoma, a common but untreatable malignancy, a better outlook. A cell line that expresses a tumor antigen recognized by T cells from most HLA-A2 positive patients with renal cell carcinoma has been genetically modified in cooperation with Thomas Blankenstein at the MDC and Dolores Schendel from the GSF in Munich. The genetically engineered cancer cell line expresses costimulatory molecules and interleukin 7 and can effectively activate cytotoxic T lymphocytes. It will be possible to overcome what has been a major limitation in cancer vaccines thus far; namely, the difficulty of monitoring the ongoing immune response in order to design powerful vaccine schedules.

A further approach that utilizes the opportunities offered by recombinant technology is the generation of bispecific-single chain antibodies. Such constructs can target T lymphocytes to cells that express a tumor-associated or a tissue-specific antigen recognized by monoclonal

antibodies. In collaboration with Gert Riethmüller (Dept of Immunology, Univ. of Munich), Robert Rössle hematologists are planning a phase I study for the treatment of lymphoma patients.

The surgical oncologists at the Robert Rössle clinic are working on improving the survival of cancer patients by improving both diagnosis and treatment. Recently 3 dimensional ultrasonography has been developed to permit characterization of the tumor extension far better than can be appreciated by the surgeons visual skills. Numerous treatment strategies are being employed to optimize outcomes. Combined pretreatment approaches including hyperthermia are currently under clinical investigation for esophageal and rectal tumors as well as for soft-tissue sarcomas. In the treatment of sarcomas and melanomas of the extremities, a surgical limb isolation provides for highly efficient hyperthermic combination therapies as a sole curative procedure or with combined neoadjuvant interventions. Cytokines, such as tumor necrosis factor are also being tested as adjuvant therapies. Finally, in the framework of the OP 2000 initiative, we are making sweeping changes in the surgeon's work place. Digital data processing, the fusion of information from multiple simultaneous sources, comprehensive virtual presentation of complex surgical data sets, robotics, and interactive consultations with colleagues outside the operating room will become routine.

Figure 6: Concept of the Berlin-Buch Campus: basic research (MDC), clinical application (RRK, FVK) and commercial (BBB) use are realized in close collaboration.



Genome Research in Berlin-Brandenburg

The Berlin-Brandenburg region is outstanding as far as German genome research is concerned. Berlin-Brandenburg is deeply involved in both the Human Genome Project as well as the Plant Genome Project and the genome-related BMBF (German Federal Ministry of Education and Research) Priority Projects. Of all the funding made available for the German Human Genome Project almost a third was won by the Berlin region. Most of the key units forming part of the infrastructure of genome research are to be found in the region: the Resource Centre in Berlin-Charlottenburg, which plays a key role in both fields, the Gene Mapping Centre of the German Human Genome Project (Deutsches Humangenomprojekt, DHGP) at the MDC as well as the Max Planck Institute of Molecular Plant Physiology in Golm. The Berlin-Potsdam area has one of the highest concentrations of biotechnology companies which are growing at a faster rate than any other sector of industry.

In order to safeguard and expand the local potential and expertise of the region and to strengthen its competitive position with respect to rival regions (particularly Munich, Heidelberg, and Cologne), it has been suggested that there should be a huge expansion of genome research in the Berlin-Brandenburg region.



Figure 7: Expansion planned for the Berlin-Buch Campus, showing (in blue) the Communications Center and the Genome Research Center.

The establishment of a Berlin-Brandenburg Centre for Genome Research will offer the following:

- coordinated activities will reinforce existing activities and promote a synergistic effect
- Berlin-Brandenburg will become a leader in German genome research
- the establishment of innovative companies will be accelerated
- the development of products with commercial potential will be speeded up
- there will be a sound foundation which will encourage talented young researchers to remain in the Berlin-Brandenburg region rather than lose them to regions with a stronger industrial base.

In 1999 a proposal for expansion was made by senior researchers at the MDC and with its support. The plan involves a laboratory for medical and functional genome research (which will have to be built) to complement the following areas: genetics and bioinformatics, functional genetics, and genetics and therapy.

Biotechnology Park

In accordance with the recommendations of the Science Council, the MDC has set up a Biotechnology Park on the Berlin-Buch Campus. In 1995, the MDC also established an offspring facility, the Biomedical Research Campus Berlin-Buch (BBB) GmbH. Co-partners include Schering AG and the Forschungsinstitut für Molekulare Pharmakologie (FMP), both of which have a 20 per cent share. Its task is to develop the Berlin-Campus and attract Biotech companies to settle in Buch.

So far, over 30 biotech companies with more than 400 employees have come to the Berlin-Buch Campus to work closely with scientists from the MDC and clinicians from the two university affiliated clinics – Robert Rössle Cancer Clinic and Franz Volhard Clinic for Cardiovascular Diseases.

In September 1998, the BBB GmbH opened a new Biotechnology and Business Development Center on the Berlin-Buch Campus. It was set up with approximately DM 30 million from the Common Mission for the Improvement of Regional Economics (GA) and the European Fund for Regional Development (German abbr. EFRE). In December 1999, the BBB GmbH laid foundations for a second new laboratory building. A production facility will also be built. Both buildings have received 19 million DM in support from the GA and EFRE. In addition, due to the great demand for space from additional entrepreneurs, a third new building for the Development Center is under negotiation with the Senate Administration for Business and Economy.

Figure 8: Inauguration of the BBB's Biotechnology and Business Development Center for start-up companies on the Berlin-Buch Campus on September 8, 1998. From left to right: Jürgen Rüttgers, the former Federal Research Secretary, Gudrun Erzgräber, Business Director of the BBB Biomedical Research Campus Berlin-Buch GmbH, Eberhard Diepgen, the Mayor of Berlin, and Peter Strieder, a member of the Berlin Senate, looking at the bust of Erwin Negelein, the Berlin-Buch scientist after whom the new building has been named. The bronze bust was the work of the Berlin sculptress Sabina Grzimek.



The Helmholtz Association

The MDC is one of 16 research institutions belonging to the Hermann von Helmholtz-Association of National Research Centres. They pursue long-term governmental research goals with complete scientific autonomy. The concentration of considerable financial and staff resources, a sophisticated scientific and technical infrastructure, and efficient management enable the Research Centres to deal with complex scientific and technical issues and interdisciplinary projects, to operate large-scale scientific and technical equipment and also to develop system solutions.

The Helmholtz Centres perform scientific and technical as well as biological and medical research and development. In the next few years, the health research activities of the Helmholtz Association will become more important and will concentrate on the great scientific and therapeutic challenges offered by medicine. In spite of the great advances that have been made in medicine, in only about a third of all known human diseases can the cause actually be treated, while the remaining two thirds receive only symptomatic treatment or cannot be treated at all. The key areas of competence of members of the Helmholtz Association in basic biomedical research, clinical research (in collaboration with external partners), medical technology and "public health" research is an absolutely crucial precondition for establishing a major scientific network which will function effectively in an interdisciplinary manner in the long-term.

The centres of the Helmholtz Association which are actively engaged in research into health set up a scientific network in 1998, "Health Research Group" in order to develop a scientific network aimed at coordinating all the health-oriented research carried out by the Helmholtz Association. In addition, via its representatives on scientific and politico-research committees, the group will actively play a part in decisions involving all health matters of scientific and political importance, even acting as an advisor when required. The Health Research Group is also an umbrella organization for the Clinical-Biological Research Group (Klinisch-Biomedizinischer Verbund, KBF), which covers in the context of the Helmholtz Association clinics as well as university and non-university institutions such that the key areas of interest of the KBF Group are much more oriented towards clinically important topics.

In the next few years all the following key areas will be investigated:

- biomedical basic research into gene regulation, cell biology, transcription control, cell-cell interaction and virus-cell interactions
- functional genome and proteome analysis, particularly by developing DNA-chip technology and protein-chip technology.
- bioinformatics as the basis for the development of a new form of theoretical biology.
- tumor immunology, protection from infection, gene therapy and new vaccination strategies.
- development of preclinical animal models

The three Helmholtz Centres, the German Cancer Research Center (DKFZ, Heidelberg), GSF-National Research Center for Environment and Health (Neuherberg) and MDC, due to their collective expertise play a key role as local sites of competence as far as the German Human Genome Project is concerned. In all biomedically oriented Helmholtz Centres, structures will also be developed to improve collaboration in clinical research, since this is the only way of effectively testing the new approaches to therapy that are developed in the laboratory.

The Helmholtz Centres receive 90 per cent of their basic funding from the Federal Government and 10 per cent from the respective host state. They have a total budget of about 4 billion DM and a staff of roughly 22,000.



Figure 9: One of the techniques students learn in the "Hands-on Laboratory" is how to isolate the genetic material DNA from tomatoes. The laboratory was opened to the public on the Berlin-Buch Campus in April 1999.

External Evaluation

Over the period November 1996 to April 1998 three external assessments were carried out at the MDC. In April 1998, under the chairmanship of Prof. Pasko Rakic, New Haven, USA, an assessment was carried out of the Structural Biology, Genetics and Neuroscience research groups. These three assessments meant that all the key research areas of the MDC were subject to external evaluation.

All three assessments were carried out in accordance with the same basic principles as used for the special research areas of the DFG. The commission of assessors visited the MDC for a two-day period during which they had the opportunity to get to know the researchers during a series of presentations made by the latter.

Following the "site visit", the commission of assessors prepared a report in which they evaluated in detail, among other things, the cooperation between the basic researchers of the MDC and their counterparts in the Robert Rössle Clinic and the Franz Volhard Clinic.

Based on the recommendations of the assessors, the MDC adopted and implemented a series of measures. For example, there was a change in the way the budget was allocated and a long-term allocation was made for the groups assessed and the organizational structure was modified to target the latest key areas of scientific interest. A further example of the changes implemented was the establishment of a service "Career Guidance for Trainee Scientists" at the MDC. These and other measures were discussed at length by the Scientific Committee of the Board of Trustees and the results of these measures are regularly monitored by means of a check list.

Graduate Student Education – Dean of Graduate Students

The support and structure of graduate student education at the MDC is of extreme importance. We have established a graduate program to accommodate highly qualified candidates and prepare them for careers in scientific research. 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 receive a broad training in the biomedical sciences. A strong student-advisor relationship is essential for outstanding academic performance and is the basis for turning 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.



Figure 10: The winners of the Max Delbrück scholarship 1997, Anja Plewinsky (2nd from left) and Judith Kreutzberg (3rd from left), being congratulated by Detlev Ganten, Scientific Director of the MDC (on the right), and Hans-Jürgen Delbrück from the Delbrück Family Foundation (on the left) on the occasion of the MDC's New Year's Reception on January 19, 1998.

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 people involved 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 indispensable 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 of this journal is WILEY-LISS, New York, USA, editors-in-chief are Bruce Ransom and Helmut Kettenmann.

International Collaborations Chinese-German Microsatellite Center in Beijing

The Chinese Academy of Medical Sciences (CAMS) and the MDC will open a joint gene mapping microsatellite center, which is currently being set up at the CAMS Fu Wai Hospital. The aim is to identify genes involved in the onset of cardiovascular diseases. The setting up of this microsatellite center is being financed by the German Federal Ministry for Education and Research. Collaborators also include clinicians from the Franz Volhard Clinic for Cardiovascular Diseases, Charité, Berlin-Buch Campus, and researchers from Hoffmann-La Roche, Basel (Schweiz).

The "Verein der Freunde und Förderer" of the MDC supports this initiative with stipends for visiting scientists. For further information on this association contact Michaela Henselmann (mhensel@mdc-berlin.de).



Figure 11: Welcome to Zhou Guangzhao, Vice-President of the National People's Congress of the People's Republic of China (in the middle), and his wife, by Detlev Ganten, Scientific Director of the MDC (on the left), on July 9, 1999.

Congresses

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

6th International Gene Therapy Symposium in Berlin-Buch

Approximately 250 scientists from the United States, Canada, France, Great Britain, Austria, Switzerland, Israel and Germany attended the 6th Gene Therapy Symposium of the MDC on May 4-6, 1998, in Berlin-Buch. The focus that year, under the title "Towards Gene Therapeutics", was basic research. New insights and knowledge arising from the development of so-called "gene vectors" and their target organs were key points of interest.

In addition to the MDC, the Medical Biotechnology Research Group of the German Society for Chemical Instruments, Technology and Biotechnology (DECHEMA) co-organized the meeting. The Symposium was accompanied by an exhibition of biotech companies. It was the last gene therapy symposium organized by the late Michael Strauss who initiated this series of conferences in 1993.

1998 Forum of European Neuroscience

The 1998 Forum of European Neuroscience was organised by the European Neuroscience Association (ENA) in collaboration with the German Neuroscience Society in Berlin from June 27 - July 1, 1998.

4,000 attended the meeting. The goal of the meeting was to integrate all National Neuroscience Societies and many European Monodisciplinary Societies and Groups. All major national neuroscience societies agreed not to hold their annual national meeting in 1998, and to support the Forum meeting. On the occasion of the 1998 Forum Meeting, FENS (Federation of European Neuroscience Societies) was founded. Helmut Kettenmann is the treasurer of FENS.

Academic Appointments

Twenty-seven group leaders have been appointed to the MDC since its foundation in 1992, five of whom were appointed in the years 1998 and 1999. The five group leaders that have joined the MDC during the last two years reported are André Reis (1998), Zoltán Ivics, Manfred Gossen, Kirsten Falk and Olaf Röttschke (1999).

Manfred Gossen

Biologist Manfred Gossen was appointed group leader at the MDC for five years in 1999. He heads the research group "Control of DNA-Replication" within the research program "Cell Growth and Differentiation". Manfred Gossen was born in Siegburg, Germany. From 1982 – 1987 he studied Biology at the universities of Bonn and Heidelberg. He gained his doctorate at the Zentrum für Molekularbiologie der Universität Heidelberg (ZMBH) in 1993. He joined the University of California in Berkeley, USA, as a post-doc in 1994 for five years before coming to the MDC.

Zoltán Ivics

The biologist and geneticist, Zoltán Ivics, was appointed group leader at the MDC for a period of 5 years in summer 1999. His field of expertise involves transposable DNA elements, transposons, that can be found in the genomes of most living organisms, from bacteria to humans. Zoltán Ivics is a native of Budapest (Hungary) and started his biological studies there in 1988. He studied at the University of Minnesota (USA) in 1991 and gained his doctorate at the University of Agricultural Sciences, Gödöllő, Hungary, in 1994. He continued as a post-doc in Minnesota until 1997. He then joined the Netherlands Cancer Institute in Amsterdam for two years before coming to Berlin-Buch.

Kirsten Falk and Olaf Röttschke

Kirsten Falk and Olaf Röttschke have been appointed joint leaders of the group "Cellular Immunology" for a period of 5 years. Kirsten Falk, born in Bremen, and Olaf Röttschke, born in Wiesbaden, studied Biochemistry at the Universities of Hannover and Tübingen. They first worked at the

Max-Planck-Institute for Biology, Department of Immunogenetics, in Tübingen and gained their doctorate in 1993. Since then, they were postdoctoral fellows at Harvard University, Department of Molecular and Cellular Biology, and started work at the MDC in 2000.

Hermann Haller, Franz-Volhard-Clinic for Cardiovascular Diseases of the Charité Medical School of the Humboldt University of Berlin and MDC, has accepted a professorship "Nephrology" at the Medizinische Hochschule Hannover and started work there in August 1999. Ludwig Thierfelder (Franz Volhard Clinic and MDC), accepted a professorship for "Genetics in Cardiology" at the Charité in February 1999.

Ingo Morano accepted a professorship at the Charité in "Molecular Muscle Physiology". Thomas Sommer, whose focus of research is the ubiquitin system and the endoplasmatic reticulum, accepted a position at the MDC. Teymuras Kurzchalia accepted a position at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden. Burkhard Micheel has become professor for Biotechnology at the University of Potsdam. Dieter Riethmacher became group leader at the Center for Molecular Neurobiology (Zentrum für Molekulare Neurobiologie der Universität Hamburg ZMNH) at the University of Hamburg.

Geneticist André Reis, head of MDC's gene mapping microsatellite center, has been offered a professorship at the University Erlangen-Nürnberg. Thomas Blankenstein (MDC) has been offered a professorship in "Immunology and Gene Therapy" at the University of Hamburg. Jürgen Behrens has been offered a professorship in "Experimental Medicine" at the University of Erlangen-Nürnberg and Reinhold Förster, in "Experimental Surgery" also at the University of Erlangen-Nürnberg. Stefan Schumacher has been offered a junior group position at the University of Hamburg.

Obituary Michael Strauss

The staff of the MDC mourn the tragic death of Michael Strauss. This internationally renowned cell biologist died on April 29th, 1999, aged 49, after a severe illness. MDC not only lost a brilliant scientist, who combined a deep commitment with a breadth of vision, but also a dear and treasured colleague.

Michael Strauss was born in Berlin on January 12th, 1950. He studied biology at the Humboldt University, Berlin, gaining his doctorate in 1977 and his post-doctoral lecturing qualification (Habilitation) in 1987. From 1981 to 1989, he led a research group at Berlin-Buch and spent several periods abroad carrying out research in Great Britain and the USA. This included work at the Imperial Cancer Research Fund and Royal Postgraduate Medical School (both in London) as well as Cold Spring Harbor Laboratory, New York (USA).

In 1992, Michael Strauss started a five year period as leader of a research group belonging to the Max Planck Gesellschaft at the Humboldt University, Berlin, which was based at the MDC. In 1993, he became head of an international research group at the Danish Cancer Society in Copenhagen and, in 1994, he was invited to become Professor of Molecular Cell Biology at the Humboldt University.

His main research interests included the regulation of cell division, the function of tumor-suppressor genes, developing gene therapy for cancer and genetically regulated conditions as well as developing viral vectors for delivering gene therapy. Michael Strauss and his collaborators in Berlin, Copenhagen and London discovered a mechanism which controls cell replication and which malfunctions in virtually all cancer cells. Using this finding, he and his colleagues developed a new approach to combat malignant diseases using gene technology. He quickly

transferred this knowledge from the laboratory bench to good manufacturing practice: he held about 25 patents and, in 1996, he set up a gene therapy company (HepaVec).

Michael Strauss received a number of honours including the Fichte Prize from the Humboldt University in 1971 and the Virchow Prize (from the GDR Ministry of Health) in 1984. In addition, Michael Strauss received fellowships from the European Molecular Biology Organization (EMBO), the Union Internationale Contre le Cancer (UICC) and the Imperial Cancer Research Fund (ICRF).

Michael Strauss was a member of many professional societies, both at home and abroad, including the American Society for Gene Therapy and the European Working Group on Gene Transfer (EWGT). In addition, he was a member of the Scientific Advisory Council of the Federal Medical Council for Somatic Gene Therapy and chairman of the "Medical Biotechnology" working group of the German Society for Chemical Instrumentation, Chemical Techniques and Biotechnology (DECHEMA).

Michael Strauss published over 80 research papers as well as about 30 reviews and book chapters. In 1993, he set up the international gene therapy symposia at Berlin-Buch and he was involved running them ever since; the last one took place in 1998.



Figure 12: The late Michael Strauss, research group leader at the MDC, in his laboratory.

Awards

A number of prestigious prizes have been awarded to scientists of the MDC and clinicians of the collaborating university affiliated Robert Rössle Cancer and Franz Volhard Clinic for Cardiovascular Diseases in 1998 and 1999.

Heinrich-Wieland Prize awarded to Thomas Willnow

Thomas Willnow, a Heisenberg scholar and research group leader at the MDC, has discovered one of the causes which lies behind the metabolic defect of a severe renal disease called Fanconi's Syndrome. He has been investigating a binding site (receptor) on the kidneys the function of which had remained unknown until recently. He was able to show that this receptor acted as a sort of collecting point for the vitamin D filtered from the kidneys. Before it is removed, the receptor, known as megalin (gr. mega = large), intercepts the vitamin D and returns it back to circulation via the cells which coat the renal tubules (epithelial cells). Now, scientists know why patients with renal disease have bone defects. For his research Thomas Willnow was awarded the Heinrich Wieland Prize worth 50,000 DM in Munich in October 1998.

Deutscher Krebspreis awarded to Walter Birchmeier and Peter M. Schlag

Monika-Kutzner-Preis der Berlin-Brandenburgischen Akademie der Wissenschaften awarded to Jürgen Behrens

For their research on the development of cancer and metastases, Walter Birchmeier and Jürgen Behrens have been awarded the Deutsche Krebspreis 1999, and the Monika-Kutzner-Preis der Berlin-Brandenburgischen Akademie der Wissenschaften, respectively. Walter Birchmeier shares the Krebspreis worth 30,000 DM with Peter M. Schlag, surgeon at the Robert-Rössle-Cancer Clinic (Charité, Berlin-Buch Campus). Peter Schlag has been honoured for the development of new therapies against intestinal cancer and soft tissue sarcomas. They received their award from the Deutsche Krebsgesellschaft in

March 1999, Jürgen Behrens received his prize worth 20,000 DM in June 1999 in Berlin.

Galenus-von-Pergamon-Preis 1999 awarded to Volhard clinicians and an MDC scientist

For their research on hypertension in pregnancy, Friedrich Luft, Hermann Haller, Volker Homuth (Franz Volhard Clinic, Charité, Berlin-Buch Campus and MDC) and Gerd Wallukat (MDC) received the Galenus-von-Pergamon-Prize 1999 (worth 25,000 DM) in November 1999 in Düsseldorf. Hypertension is one of the most common complications of pregnancy. It can interfere with the development of the fetus and can lead to premature termination of the pregnancy. Hypertension due to pregnancy is accompanied with the excretion of albumin in the urine after the 20th week. The reasons for this condition, known as pre-eclampsia or gestosis (lat. gestare – to carry), are unknown. During their research the clinicians found clues as to the cause of this serious condition. In a collaborative effort with Gerd Wallukat (MDC), they discovered autoantibodies in the blood of pregnant women suffering from pre-eclampsia. These autoantibodies bind to the receptors for Angiotensin II, a hormone that plays a role in regulating blood pressure. This activates the receptors and leads to an increase in blood pressure. It is not yet known what causes the formation of these

autoantibodies. The clinicians suspect that the trigger for this comes from the fetus since after delivery there is no longer any sign of these autoantibodies in the blood of women who had pre-eclampsia.

Innovationspreis Berlin/Brandenburg awarded to Dr. Regina Reszka

Regina Reszka from G.O.T. GmbH & Co. KG and the MDC was awarded the Innovationspreis Berlin/Brandenburg 1998 worth 100,000 DM shared with three other companies in November that year. She received the prize for the development of a "universally" applicable non-viral gene transfer system (a cationic derivative of cholesterol, DAE-Chol liposomes). This system is intended to be used for the gene therapy of cancer, especially brain – and liver tumors, and against stenoses. It has been developed by Detlef Groth, Jana Richter and Ingrid Berger (all MDC) of Regina Reszka's group.

Figure 13: Lothar Weißbach, President of the German Cancer Society (on the right), handing over the "Deutsche Krebspreis 1999" to Walter Birchmeier (MDC) and Peter M. Schlag (Robert Rössle Cancer Clinic, Charité, Berlin-Buch Campus, and MDC) in Heidelberg on March 24, 1999.



InnoRegio-Competition: Berlin-Buch reached the final round

In the competition announced by the Federal Ministry for Education and Research (Bundesministerium für Bildung und Forschung, BMBF) for structurally less developed regions in the new German states, the InnoRegio, the Gesundheitsregion Berlin-Buch e.V. reached the final round. In November 1999, an independent jury in Berlin chose from the 444 regions that had entered a total of 25 for this round and the Berlin-Buch health region was one of these. The winners have eight months to develop their ideas into workable projects and each of these regions will get up to 300 000 DM from the BMBF. The final decision will be made in Summer 2000 and the projects will be implemented before the end of 2005. The BMBF will provide 500 million DM in funding for this. The "Gesundheitsregion Berlin-Buch e.V." hopes that this InnoRegio programme will provide their region with additional funding for the areas of Berlin-Buch, Karow, Blankenburg, and Heinersdorf as well as the Panketal Office in Brandenburg. In the next few years there will be a "future-oriented" total investment of almost a billion DM to help allow the region to become an attractive site for all aspects of healthcare on scientific, economic, artistic, and cultural grounds as well as being a provider of health-associated services. The aim is to

attract jobs to the region and generate new employment opportunities. A group was set up to pursue this goal in July 1999 consisting of clinicians*, researchers from the MDC and the Forschungsinstitut für Molekulare Pharmakologie (FMP), biotech companies on the Berlin-Buch Campus, teachers, self-help groups and socio-cultural bodies as well as the Academy of Arts in Berlin-Buch and the Barnim district. The group is chaired by Jens Reinwardt, manager and head of the School for Health Professions e.V.

**Representatives of the Berlin-Buch clinics, including the city, private sector, universities and other public bodies.*



Figure 14: Edelgard Bulmahn (3rd from right, front row), Secretary for Science and Education, congratulates the Berlin-Buch "InnoRegio" team on its success in the InnoRegio Competition set up by the Federal Ministry to develop East German regions.

Genetics, Bioinformatics and Structural Biology

Genetics, Bioinformatics and Structural Biology

Research in molecular genetics is currently undergoing a fundamental change triggered by the rapid progress of genome sequencing projects, notably the Human Genome Project. Landmark events such as the completion of the human chromosome 22 sequence or the announcement of a first working draft of the human genome sequence by the Spring of 2000 testify to the pace and vigor of this collaborative international project. At the same time, concepts for science in the post-genome era are gaining clearer contours. These concepts are directed towards obtaining a comprehensive and general view of processes including protein synthesis and processing, signal transduction from the cell surface to the nucleus, cell differentiation, or the development and function of entire organs. These topics are often summarized under the term functional genomics. Proteomics, aiming at establishing the complete cellular protein inventory in a defined physiological or pathophysiological state, or structural genomics, an effort to map the entire protein "universe" at the level of protein domain folds, are important components of functional genomics. MDC scientists are aware of the relevance of genome research and functional genomics to molecular medicine. We have already taken the first initial steps and are determined to move into these fields in the near future.

The research program Genetics, Bioinformatics, and Structural Biology combines groups active in very different areas of research from patient-oriented genetics, genetic field working and genotyping projects to experimental and theoretical studies of macromolecular folding and structural characteristics. The connecting theme of these research endeavors is that

together they provide the core expertise for research into functional genomics. Crucial methodological approaches in that context include animal models for studying gene function and disease pathways, as well as bioinformatics to evaluate genomic sequences and disease-related genetic variations. Part of the research in structural biology is devoted to pilot projects in the areas of proteomics and structural genomics.

Important insights into the pathogenesis of human disorders can be obtained by studying animal model systems. In hypertension and vascular research, multiple non-transgenic and transgenic rodent strains offer the opportunity to identify causative or modifying genetic components of various disease phenotypes. Detlev Ganten's group is spearheading efforts at MDC to generate rat genome tools (genetic and physical maps, genomic and cDNA libraries etc.) necessary for the identification of genetic abnormalities in the rat. The generation and molecular characterization of congenic rat strains should ultimately allow fine mapping and identification of complex genetic traits.

Three groups in the program employ mice as model organisms to study the function of genes, using targeted mutations, conditional mutations or other YAC transgenic technologies.

Thomas E. Willnow is analyzing the low density lipoprotein (LDL) receptor gene family and the role of these gene products in the physiology and pathophysiology of lipid metabolism and other disorders. By generating mice that lack the gene for the LDL receptor, the LDL receptor-related protein (LRP) or both, his group has shown that the clearance of

dietary lipids proceeds *via* a dual lipoprotein receptor system, the LDL receptor and LRP. Megalin, another member of the LDL receptor gene family, has been found to act as an endocytic receptor for the uptake of lipophilic vitamins and regulates transport and renal conversion of vitamin D₃ metabolites.

Andreas Schedl is analyzing the Wilms' tumor gene (WT1) that, when mutated, causes Wilms' tumor, a common childhood malignancy, and the Frasier and Denys-Drash Syndromes, which are characterized by abnormal gonadal development. WT1 plays a crucial role in renal development. Following analysis of YAC transgenic mice, WT1 has been shown to be required continuously during nephrogenesis, in particular, during the formation of mature glomeruli.

Carmen Birchmeier's group has found that the EGF-like factor, neuregulin and its receptor, erbB2, play a dual role during the expansion of the Schwann cell precursor pool and during myelination. Moreover, the group has elucidated the function of cryptic, another EGF-like factor, that has been found to be essential for establishing the left-right axis. Cryptic mutant mice display laterality defects, such as malposition of the great arteries, right isomerism of the lung and splenic hypoplasia. This phenotype is reminiscent of the asplenic syndrome in humans that is typically associated with laterality defects.

Further development of miniaturized technology for a more detailed characterization of whole animals, isolated organs, and functional units will be required for a better understanding of pathophysiological disease pathways in rodent models. Friedrich Luft's group has developed sophisticated tools to study rodent models *in vivo*. His group, as well as the groups of Michael Bader and Ludwig Thierfelder have applied these tools to the characterization of rodents with various genetic cardiovascular modifications.

Although rodents and other animals can be extremely useful in elucidating disease pathways, the ultimate model system for human diseases is man. Great progress has been made in recent years in the molecular characterization of single gene

disorders. Various MDC groups have contributed in the elucidation of monogenic diseases, including André Reis and Herbert Schuster. The current scientific challenge is to delineate the genetic components of complex traits. Extensive genetic field working facilities established by Herbert Schuster, a gene mapping center with a capacity to generate 2,000,000 genotypes per day led by André Reis, and the multiplex sequencing technology for the identification and typing of single nucleotide polymorphisms, established by Margret Hoehe, will provide a framework for the characterization of complex genetic diseases in humans.

The group of Siegfried Scherneck is studying genetic susceptibility factors for breast cancer. His group has analysed the genes of German families that have a high risk of developing breast cancer. The majority of the families investigated carry mutations in known cancer susceptibility genes. However, every fourth family studied does not carry these mutations and a novel breast cancer susceptibility gene has been located on chromosome 8. Moreover, the group participates in a nationwide program initiated and supported by the "Deutsche Krebshilfe". This endeavor offers women options for risk calculations, genetic counseling and provides clinical and psychological support.

Important links between the genetics and structural biology components of the program, as well as between MDC-based basic science and patient-oriented research at the clinics, are provided by the Bioinformatics Unit. At present, the group operates at two sites with a genetics section in Berlin (Jens Reich) and a biocomputing section at EMBL, Heidelberg, where Peer Bork is currently a visiting scientist. Combining both approaches, the bioinformatics unit is evaluating variations in the human genome that are relevant to the diseases treated and investigated at the Franz Volhard Clinic (lipoprotein disorders, arteriosclerosis and hypertension, with Friedrich Luft) and at the Robert Rössle Clinic (tumor and pertinent normal tissue, with Peter M. Schlag).

The Structural Biology groups of MDC use a wide range of experimental techniques to study problems of protein and nucleic-acid

structure, folding, dynamics and function. Together with complementary methods offered at the Forschungsinstitut für Molekulare Pharmakologie (FMP), they provide the Buch Campus with expertise to address nearly all aspects of structural biology relevant to medicine and pharmacology. In the Berlin Brandenburg area, these groups are integrated in and provide leadership for the Koordinationszentrum Strukturforchung (KoSt), an organization supported by the Senate of Berlin. The aim of KoSt is to coordinate structural analyses of a wide range of objects, from biomolecules to shapes, surfaces and textures important in the materials sciences. Responding to the challenges posed by the international genome programs, a Berlin-based initiative has begun to set up a structural genomics infrastructure for the high-throughput structure analysis of proteins following the sequencing of their genes or cDNAs within the German human genome project. This initiative, the "Proteinstrukturfabrik", is coordinated at MDC and funded by the BMBF.

Within the structural biology program of MDC, four main lines of research are being followed. (1) The analysis of the three-dimensional structure of proteins and nucleic acids by X-ray diffraction methods is the central theme of Udo Heinemann's research. Recent projects of his group have addressed problems of specific protein-RNA recognition, electron transfer by [2Fe-2S] ferredoxins in cytochrome P450 systems, *in vivo* folding of engineered glycosyl hydrolases, sex steroid transport in plasma by the sex-hormone binding globulin, and blood coagulation mediated by tissue factor, a member of the cytokine receptor superfamily. Computer simulations of nucleic acid structure and ligand binding (Heinz Sklenar) provide valuable information where experimental data are not available or are inaccessible. Using new algorithms for the treatment of solvent electrostatics, molecular simulations have been applied to the functional analysis of gene regulatory DNA sequences, the characterization of non-canonical structural motifs in RNA, and a binding study of singlet-oxygen generating dyes to DNA. (2) Protein misfolding events and the resulting aberrant protein conformations have received considerable attention recently due to their relevance to amyloidoses, a family of diseases

characterized by deposits of β -stranded protein aggregates in tissue. Applying a variety of experimental techniques, the groups of Gregor Damaschun and Heinz Fabian are studying the folding pathways and kinetics of a number of model polypeptides to help shed light on productive folding and pathological misfolding of proteins. (3) To fulfill their diverse physiological functions, proteins interact with many ligands. These ligands vary widely in size from small molecules to cellular structures. The binding events are characterized by very different time scales and association constants. Time-resolved Fourier-transform infrared spectroscopy is being used to study structural changes and reaction intermediates associated with electron transfer in cytochrome P450 (Christiane Jung). Antibody-peptide interactions, the specific binding of the tetracyclin repressor to operator DNA and initiator-tRNA binding by the initiation factor IF2 are being investigated by circular dichroism, fluorescence, infrared and Raman spectroscopy as well as calorimetric methods in Heinz Welfle's laboratory. Finally, analytical ultracentrifugation is being employed by Joachim Behlke to study the interaction of protein domains with unusually structured DNA, protein oligomerization and the nucleation of protein crystal growth. (4) In a large number of collaborations with extramural and MDC groups, protein chemistry, peptide sequencing and mass spectrometry (Brigitte Wittmann-Liebold) have proven to be indispensable tools for modern molecular and cell biology research. In addition, highly sensitive protein 2D-electrophoresis combined with MALDI mass spectrometry are key techniques in proteome research where the goal is to establish protein patterns characterizing cellular states, such as apoptosis, or processes such as cell differentiation and development.

Carmen Birchmeier, Udo Heinemann, Friedrich C. Luft, Jens Reich, Ludwig Thierfelder

Molecular Biology and Genetics of Cardiovascular Diseases

Detlev Ganten

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 genetic investigation is substantial.

In recent years we have demonstrated that multiple chromosomal loci in rat models contribute to blood pressure regulation and hypertension. Independent from elevated blood pressure, additional genetic factors contribute to end-organ damage and stroke in these animals.

Ongoing research in our laboratory is directed towards the identification of the underlying predisposing genes and the subsequent identification of their molecular variants responsible for different cardiovascular disease phenotypes.

To localize disease genes within chromosomal regions linked to quantitative traits (e.g. blood pressure), we are establishing multiple congenic rat strains. These congenic strains are being developed by introgressing disease alleles encompassing the quantitative trait locus (QTL) into a non-affected reference strain by successive backcrossing and molecular analysis. This strategy allows observation of the effect and 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.

Combination of congenic experimentation with the development of subcongenic animals, having only a fraction of the initial congenic segment, will allow 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 in national and international rat genome projects, our group has produced various genomic 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 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. (for more details visit our webpage: <http://www.mdc-berlin.de/ratgenom/>)

The mapping efforts of complex cardiovascular traits by congenic experimentation and positional cloning will be used in ongoing projects in combination 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 potential candidate genes within chromosomal regions for genetically determined cardiovascular diseases.

Transgenic rat technology

In order to study the functional relevance of genes linked to hypertension and stroke, transgenic rats are being 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 in understanding the physiological functions of local renin-angiotensin systems in tissues. Furthermore, transgenic rats carrying the human renin and angiotensinogen genes are excellent models for studying hypertension-induced end-organ damage, particularly in the kidney. In addition, numerous other transgenic rat models for the study of cardiovascular physiology have been produced and analyzed in collaboration with other groups. Furthermore, transgenic technology in the rat has been extended by the generation of transgenic animals with large genomic constructs and the establishment of knockout technology for this species.

Selected Publications

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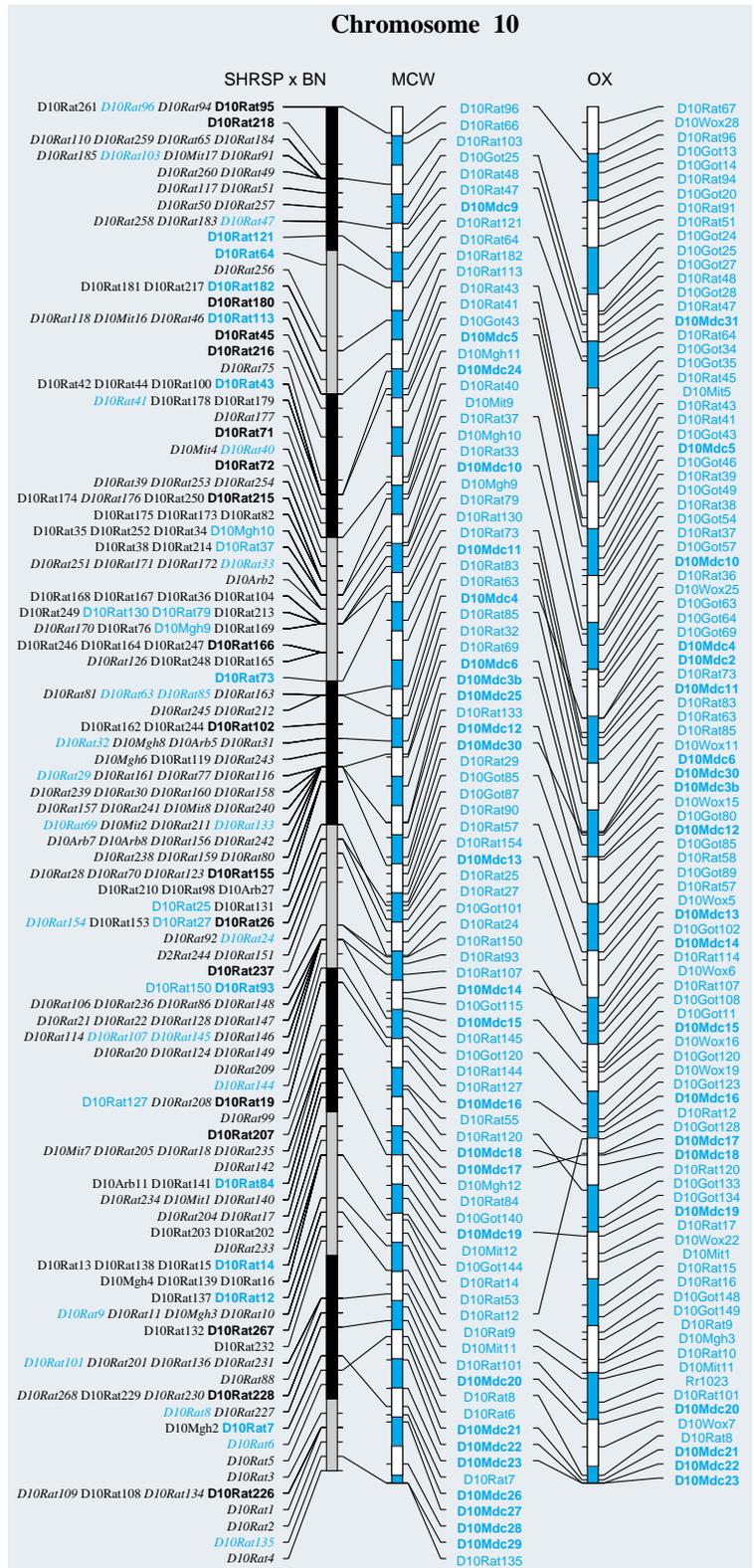


Figure 15: Integrated genetic and radiation hybrid map for rat chromosome 10. Information of the high density genetic map based on genotyping results derived from 48 animals of a BNxSHRSP intercross has been integrated with data from two available radiation hybrid framework maps that have been established by collaborating groups from Milwaukee, USA (MCW) and Oxford, UK (OX), respectively.

These maps consist of genetic markers produced in Berlin (MDC) and other laboratories and enables selection of markers for further investigation of chromosomal regions of interest. Equivalent maps have been established for all rat chromosomes and will be made available via the WorldWideWeb.

Molecular Biology of Peptide Hormones

Michael Bader

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

Renin-angiotensin system

The renin-angiotensin system (RAS) plays a key role in blood pressure regulation and, therefore, has been studied in detail employing 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 astrocytes of the brain have been produced and show a decreased local concentration of this protein and reduced blood pressure and plasma vasopressin levels. These animals are suitable models for studying the function of local angiotensin production in the brain.

In order to investigate the function of the mas protooncogene, a receptor expressed in the brain and thought to be involved in the behavioural effects of angiotensins, we produced mice lacking this protein by homologous recombination in embryonic stem cells. Mas-deficient animals develop normally and exhibit normal blood pressure and fertility. However, long-term potentiation in the hippocampus as well as anxiety behaviour is significantly altered. Furthermore, the animals show modified rhythms of blood pressure and heart rate. 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 have a targeted disruption of the angiotensinogen gene 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 angiotensin levels, the animals are hypertensive but suffer less damage to the target organs as a result of a 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 for studying the functions of the KKS in an intact animal, transgenic rats were produced

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 rhythmicity, as measured by telemetry, are 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 unknown and so we produced mice lacking this subtype. The resulting animals exhibited analgesia and altered inflammatory reactions demonstrating an important role of the B1 receptor in pain transmission and inflammation.

Embryonic stem cell technology

Using embryonic stem cell technology, the gene for the cardiac fatty acid binding protein has been deleted by homologous recombination. The knockout mice exhibit a severe defect in long-chain fatty acid utilization causing exercise intolerance and cardiac hypertrophy. Recently, we have started a series of projects to analyze the serotonin system by transgenic technology. Firstly, we have been able to show that embryonic stem cells as well as mouse blastocysts express the key enzyme in serotonin synthesis, tryptophan hydroxylase, implying an important role for this hormone in early embryogenesis. Mice lacking tryptophan hydroxylase have been produced and their genotype is under investigation.

We are also establishing embryonic stem cells from rats to allow gene-targeting experiments in this species which is more suitable for research on cardiovascular diseases than the mouse.

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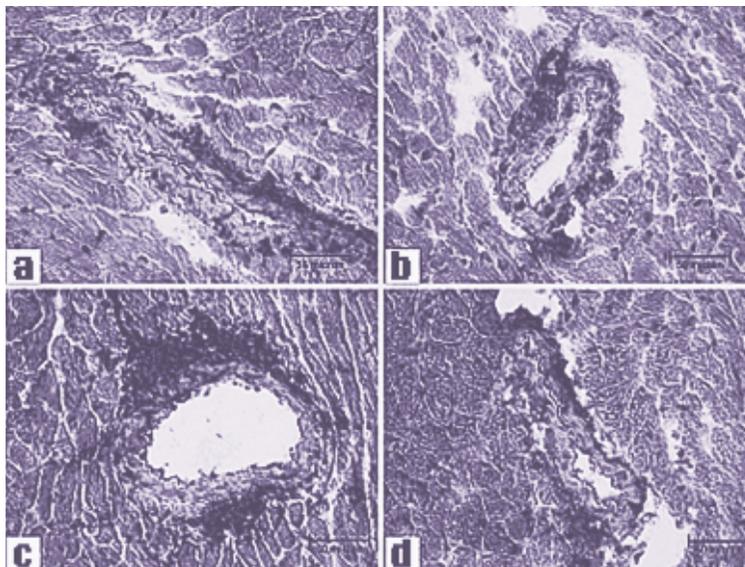


Figure 16: Role of locally produced angiotensin II in cardiac fibrosis
Mice carrying a rat angiotensinogen transgene develop hypertension, cardiac hypertrophy and fibrosis as detected by immunostaining for collagen (c). Breeding these mice with animals lacking the endogenous angiotensinogen gene results in animals which are equally hypertensive but lack local angiotensin production in kidney and heart. In these animals (b) perivascular collagen deposition is reduced to levels in normal mice (d) or angiotensinogen-deficient mice (a). These results show that hypertension-induced cardiac fibrosis depends on local angiotensin synthesis.

Genome Research, Gene Variation, and Complex Disease

Margret Hoehe

The systematic analysis of DNA sequence variation in biomedically relevant genes is the key to a) the identification of genetic risk factors in common, complex diseases ('Medical Genomics'), and b) the identification of genetic variation involved in individually different drug responses ('Pharmacogenomics'). To this end, the Genome Research Group has a) developed highly efficient technologies, which allow sequence comparisons of candidate genes in large numbers of individuals in the megabase range (e.g. 'Multiplex Sequence Comparison'); b) applied these technologies to variation analyses of candidate genes defined by biology and/or genetic mapping; c) identified numerous variants in genes potentially involved in hypertension, substance dependence, and other common, complex diseases; d) predicted numerous individually different forms of the genes by means of haplotype analyses; e) developed bioinformatic approaches to haplotype classification into functionally related groups; f) identified variants, or combinations of variants (pattern), associated with complex disease. Thus, combined approaches have been developed to establish complex genotype-phenotype-relationships against a background of high natural genome sequence variability. This line of research, development and production has been supported by the German Human Genome Project. It has prepared the background for functionally characterizing genetic variations conferring risk of disease; a 'Functional Genomics' research project is presently being established in this group.

Gene sequence diversity, haplotypes, and genotype-phenotype-relationships

We have applied the approaches described above to test the potential involvement of the human mu opioid receptor gene (OPRM1) in substance dependence. All functionally relevant regions of this candidate gene, including 6.7 kb regulatory, exonic and critical intronic sequences, were analysed by 'Multiplex Sequence Comparison' in 250 subjects and controls. A total of 43 variants were identified, and 52 different haplotypes predicted in the subgroup of 172 African-Americans. These haplotypes were classified by hierarchical cluster analysis into two functionally related categories, one of which was significantly more frequent in substance-dependent individuals. Common to this category was a characteristic pattern of sequence variants, which was associated with several forms of substance dependence (opioid and cocaine dependence). This study provides the first example of the possibility of establishing genotype-phenotype-relationships in a situation of abundant gene sequence variation. Moreover, to our knowledge, this work represents the largest body of sequence data so far on multiple individuals for the same gene (manuscripts in review). A large sample including 250 Israeli substance-dependent individuals and controls has also been analysed, and a global survey has been performed.

Systematic comparative sequence analysis of the human beta2 adrenergic receptor gene, including its known regulatory and coding regions in more than 400 individuals, resulted in a total of 15 identified variants, several of which were functionally significant. An additional 700 individuals were genotyped and these included hypertensive patients, individuals characterized by salt-sensitivity/resistance, beta2 receptor binding, vasodilator response, and a series of other cardiovascular parameters including responsiveness to various forms of experimentally induced mental and physical stress, as well as obese patients. Three major haplotypes of the beta2 adrenergic receptor gene were identified, and observed in 80-95% of all subjects from several independent studies. Evidence of a genetic risk profile for essential hypertension has been

obtained. Generally, evidence of the involvement of beta2 variation in increased blood pressure, *in vivo* vasodilator response to beta2 agonists, catecholamines, and heart size was obtained. Beta adrenergic receptor gene haplotypes are being expressed and functionally characterized. An additional technological development has led to the first application of MALDI-TOF mass spec for beta2 genotyping.

Systematic analysis of genetic variation in three chemokine receptor genes (BLR1, BLR2, and the Fusin gene) in more than 200 patients suffering from tumors such as acute leukemia, Hodgkin- and non-Hodgkin lymphomas, and controls, again resulted in numerous variations. Variants that cause an exchange of conserved amino acids have been identified, and are now being expressed and functionally characterized. Additional genes studied include the cannabinoid receptor gene, the TRHR gene, the beta1 adrenergic receptor gene, and the beta myosine heavy chain gene.

These projects have been carried out in close collaboration with the Max Planck Institute for Molecular Genetics (Berlin), Department of Genetics, Harvard Medical School (Boston), Department of Genetics, Yale University (New Haven), Franz Volhard Clinic and Robert Rössle Clinic at the MDC, Free University (Berlin), University of Graz, INSERM (Paris and Strasbourg), Karolinska Institute (Stockholm), and Pennsylvania State University (Philadelphia).

Technology transfer

Based on the fundamental research component in the 'Genome Research Group', and as a direct spinoff from the German Human Genome Project, a genome research company, GenProfile AG, was founded in September 1998 with M. Hoehe and R. Zettl as the executive board. The company is based at the Biomedical Research Campus Berlin-Buch, Germany, with research facilities occupying approximately 900 square meters. The company's main aim is the systematic identification of the molecular diversity within the human genome. Special emphasis is placed on the functional significance of this variation for the pathogenesis of human complex diseases ('Medical Genomics') and the efficacy of drugs ('Pharmacogenomics'). The company has established a powerful technology platform, in particular proprietary high-throughput technologies for comparative genome analysis as well as appropriate bioinformatic strategies for data interpretation. With a total of about DM 12 Mio. from its first round of financing, GenProfile AG has been the largest direct spinoff from the German Human Genome Project (funded by the Federal Ministry of Education and Research, BMBF). GenProfile AG has recruited 3i (Investors in Industry) Group plc, London, Europe's leading venture capital company, as lead investor. GenProfile AG has also recently been awarded about DM 4 Mio. of funding from the BMBF BioChance Project. More than thirty posts have been created.

Selected Publications

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Etiology and Pathogenesis of Hypertension and Vascular Disease

Friedrich C. Luft

This group is primarily interested in hypertension and the effect of elevated blood pressure on the vascular wall. The focus has been on animal physiology and vascular injury. Dominik Müller leads a team concentrating on unique transgenic rat models of hypertension. The focus here is to elucidate pathways resulting in end-organ damage. Volkmar Gross has focused on establishing sophisticated physiological mouse models because of the potential they offer in terms of studying gene-targeted conditions. In addition to elaborate renal function tests, he has successfully conducted telemetry measurements of blood pressure and heart rate in conscious mice. The group has a broad interest in patient-oriented research. Jens Jordan has established a laboratory for studying human vascular regulation. With microdialysis, microneurography, and sophisticated autonomic pharmacology, he is identifying disease mechanisms as well as making clinical diagnoses.

Pathophysiology of hypertension and vascular disease in animal models

Dominik Müller is interested in the putative “tissue” renin-angiotensin system. He has focused on rats harboring both the human renin and angiotensinogen strains. This model was established at the MDC by Detlev Ganten’s group. The rats develop severe hypertension and die from renal and cardiac failure, beginning at the seventh week of age. Focal necrosis, increased matrix production, fibrinoid necrosis, leukocyte infiltrates, and microthromboses in the kidney and the heart are hallmark features of this model. Together with Eero Mervaala, Joon Keun Park, Ralf Dechend, and Anette Fiebeler, the team has traced a pathway of events involving reactive oxygen species, MAP kinase activation, NFκB and AP1 activation, adhesion molecule and MCP-1 expression, and tissue factor production. With a novel set of pharmacological studies (see figure), the team has shown that endothelin is involved, that the Rho pathway seems to play a role, and that IκB kinase β must also be activated. Their findings could lead to a series of novel pharmacological interventions, above and beyond blockade of the renin-angiotensin system.

Ning Ling Kang, a doctoral student of Hermann Haller and Friedrich C. Luft, has studied streptozotocin-induced diabetes in a rat model and found that protein kinase C isoforms were differentially regulated in the kidney and heart in diabetes. High glucose increased PKC alpha expression, whereas PKC zeta was down-regulated. The finding that PKC alpha is mostly increased in endothelial cells supports a role for PKC alpha in functional endothelial disturbances observed in diabetes.

Volkmar Gross has continued his successful development of physiological techniques in the mouse. He has studied pressure-natriuresis in DOCA-salt-induced hypertension and, more recently, observed that lovastatin lowers blood pressure and restores normal pressure-natriuresis by influencing medullary blood flow in the mouse. He and Anna Francka Milia have perfected a system for 24 h telemetric monitoring in the mouse and have conducted a series of studies defining the reasons for high blood pressure in AT2

receptor knockout mice. Volkmar Gross has also established a productive collaboration with Wolf-Hagen Schunck and they are now exploring P450 enzyme-related changes in the kidney in response to bezafibrate which stimulates 20-HETE and 11,12 ETE production. Finally, collaboration with Thomas E. Willnow’s group involves the characterization of renin binding protein knockout mice

Subject and patient-oriented research (POR)

Jens Jordan has rejoined the group after completing a fellowship in clinical pharmacology at Vanderbilt University. He is interested in the autonomic regulation of blood pressure and cardiovascular reflexes. Recently, he studied subjects with monogenic hypertension and brachydactyly. These subjects all exhibit an aberrant loop of the posterior inferior cerebellar artery, which impinges on the ventrolateral medulla. Such loops have been implicated in hypertension by putatively interfering with baroreceptor reflex function. Jens Jordan, Jens Tank, and others have studied these subjects systematically and observed that they exhibit orthostatic hypertension and do not buffer increases in blood pressure by reducing sympathetic tone. Microneurography, microdialysis, and a battery of autonomic tests, including ganglion blockade, are the techniques most favored by this team.

Selected Publications

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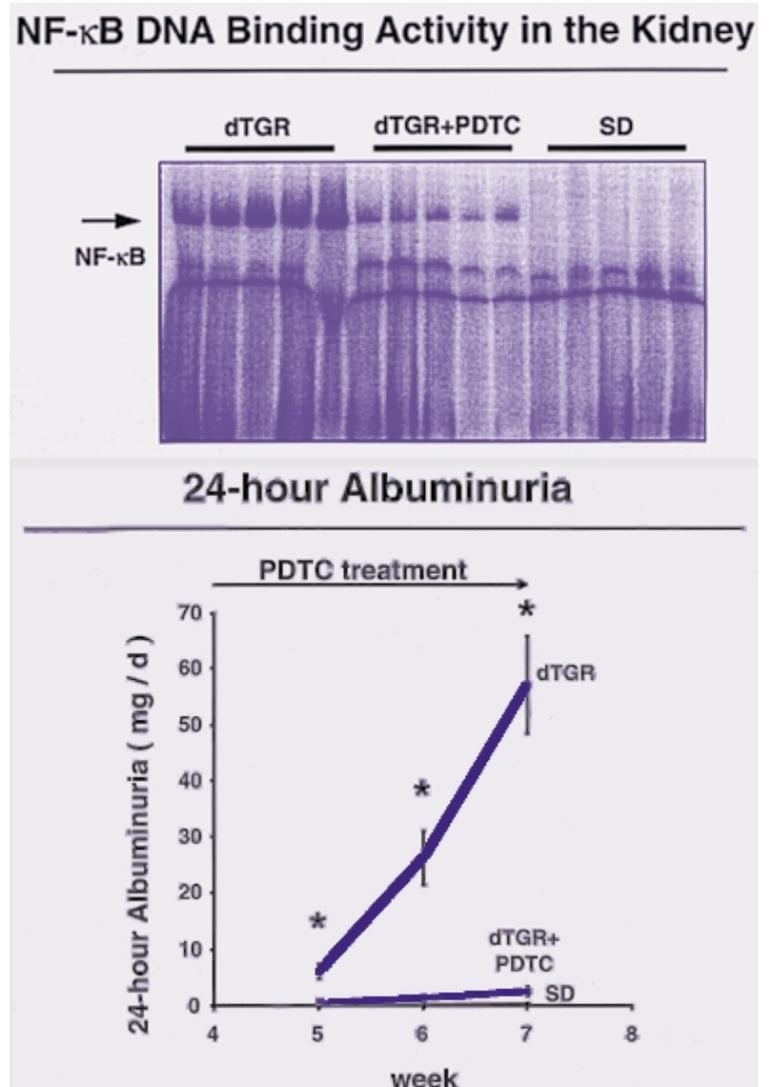
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Figure 17: Electrophoretic mobility shift assay for the transcription factor NF κ B in kidney from double transgenic rats, double transgenic rats treated with PDTC an NF κ B inhibitor, and control rats. The lower panel shows proteinuria, which is almost completely blocked when NF κ B activation is inhibited.



Gene Mapping and Identification in Monogenic and Complex Diseases

André Reis

Positional cloning is now widely used for the identification of gene defects that underlie inherited diseases. A necessary first step for positional cloning is the mapping of the gene locus that co-segregates within families with a particular disease or trait, which allows allocation of a specific chromosomal position to the responsible gene. Although mapping was initially developed for monogenic traits, it is now possible to locate genetic factors involved in the aetiology 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 Centre is a specialised laboratory carrying out 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 reference genetic map with different marker densities to accommodate the requirements of special study designs. The laboratory is mainly funded through grants from the German Ministry of Science, Research and Technology (BMBF) and, since January 1997, we have participated in the German Human Genome Project. Additional funding is provided through a strategy-fund project, "genetics of complex diseases", from the Helmholtz Society of National Research Centres. The laboratory is also available for mapping projects by other groups.

Mapping of complex diseases

The main focus of the Gene Mapping Centre is mapping genetic factors in complex diseases. This type of study involves the analysis of large numbers of phenotypically well characterised families. Hundreds of markers are used for genotyping and sophisticated biostatistical analyses are subsequently required to identify the genetic loci that contribute to a complex disease. For this purpose we have established suitable techniques with an emphasis on automation of the experimental procedures. In 2000 we expect to reach an annual capacity of 2.000.000 genotypes. Currently, mapping is based on highly-informative microsatellite markers but in the future analysis will shift more towards single nucleotide polymorphisms (SNPs). Two scientists are involved in project management, genotyping and technology development. One scientist concentrates on laboratory information management (LIM) which involves the integration of genotype and phenotype data and the biostatistical analysis of these data. This is done in close collaboration with the bioinformatics group (Dr. K. Rohde) and the University of Bonn (Prof. T. Wienker) where data analysis is carried out.

A total of four genome scans for complex diseases have been completed and two further studies are under way. In a German collaborative study on the genetics of asthma (Wjst et al. 1999) 100 families with two affected siblings each, totalling 400 subjects were investigated. Furthermore, in a study to identify genetic factors for susceptibility to psoriasis, 32 extended families with three or more psoriasis patients with in all, 500 subjects were investigated (Lee et al., in preparation). Each study required a total of approx. 200.000 genotypes. Besides confirming already known loci in both studies, we have identified novel susceptibility loci, which are currently being investigated further with refined mapping and testing of positional candidate genes.

Data analysis of two studies, for which we recently completed genotyping, is well advanced. In a study of the genetics of a subtype of schizophrenia we were able to identify new susceptibility loci and determine the mode of inheritance, at least for this subtype. The largest study in our

laboratory to date is a European collaborative study of the genetics of juvenile myoclonic epilepsy. In total, we have investigated 130 families comprising 700 subjects. This is the first comprehensive genome-wide study of this type of epilepsy and we expect to gain important insights into the aetiology of both disease groups.

Ongoing studies include two affected sib-pair studies, one on atopic dermatitis from a European consortium headed by the Charité Hospital (Prof. Wahn) and a second on juvenile obesity in collaboration with the University of Marburg (Prof. Hebebrandt). Finally, genotyping of a study of the genetic factors involved in hypertension, in collaboration with the Franz-Volhard-Clinic on the campus (Prof. Luft), is scheduled for the year 2000. The study design is based on isolated populations and takes advantage of the restricted genetic heterogeneity in these populations. Running costs for all the studies are funded through additional external grants.

Mapping of monogenic diseases

In contrast to complex diseases, mapping of monogenic traits requires much less genotyping and, usually, it is sufficient to analyse 30-50 subjects. The statistical evaluation is different and often requires skilled interpretation e.g. haplotyping. In the four years since the lab opened, a total of 31 monogenic traits have been mapped in humans. For several of these the underlying gene defect has already been identified, completing the process of positional cloning. For instance, the gene for an autosomal recessive condition, Nijmegen Breakage syndrome, was found to be caused by mutations in a protein involved in DNA double-strand repair (Varon et al. 1998). This important finding has solved a long-standing puzzle and pointed research in this field in a new direction. 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 two cases, the underlying mutations have also been identified. The majority of these projects originated in external laboratories in Germany but also from England, The Netherlands, Canada, United Arab Emirates and Australia.

Selected Publications

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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 cancer (BC). BC has been shown to be caused by a multi-step process in which a number of oncogenes and tumor suppressor genes contribute to the cancer when their function is altered. Most gene mutations have a somatic basis; germline mutations in the BRCA1/2 genes make individuals more susceptible to tumorigenesis and mainly occur in hereditary BC. There is strong evidence for the existence of other BC (susceptibility) genes. We are searching for BC families and are using experimental models to identify such new genes. Molecular probes will allow us to perform genetic screening not only for those who are considered to be at higher risk but also for the general population. Precise diagnosis of BC by genetic investigation may provide useful information for choosing methods of treatment and developing new therapeutic strategies.

Detection and characterization of germline mutations in families with a high incidence of breast cancer

W. Hofmann, E. Claßen, D. Horn, L. Estevéz-Schwarz, B. Jandrig, H. Zeidler, I. Sümnick in cooperation with 11 centers for Familial Breast and Ovarian Cancers in Germany

Hereditary breast cancer accounts for 5 – 10 % of all breast and ovarian cancers that occur in the western world. Mutations in 2 genes, BRCA1 and BRCA2, jointly explain the large majority of families with breast-ovarian cancer syndrome. Using a variety of techniques, we have identified more than 30 different BRCA1-, 15 BRCA2 germline mutation and some 40 polymorphisms in about 200 German families with a high risk of BC. At present, we are participating in a nationwide, interdisciplinary approach (gynecological oncology, human genetics, molecular biology, psychotherapy), initiated and supported by the “Deutsche Krebshilfe”, to offer women options for risk calculation, genetic counseling and to provide clinical and psychological support.

Genetic heterogeneity in hereditary breast cancer: Linkage analysis and the search for further breast cancer susceptibility genes

B. Jandrig, S. Seitz, A. Nothnagel, K. Poppe, K. Rucker, S. Hahn in cooperation with K. Rohde, B. Hinzmann, A. Rosenthal, Centers for Familial Breast and Ovarian Cancers in Germany and several European and American groups organized in the Breast Cancer Linkage Consortium (BCLC)

Hereditary breast cancer has a heterogeneous genetic basis. We assessed the contribution of BRCA1, BRCA2 and other genes to hereditary breast cancer by linkage analysis in more than 100 German families. Overall, an estimated 50 % of families showed linkage of the disease to BRCA1, 25 % to BRCA2 and 25 % to other genes. Families with at least 4 cases of breast cancer were chosen for BRCA1/2 mutation analysis and mutations were detected in approximately 50 % of these families. At present, about 30 informative

families have tested negative for BRCA1/2. To investigate the role of candidate genes and/or candidate genome regions in hereditary breast cancer, BRCA1/2-negative families were used for mutation analysis as well as linkage- and association studies. We have performed linkage analysis in two BRCA1/2-negative families using microsatellite markers from the chromosome region 8p12-p22. A maximum cumulative lod score of 2.41 was obtained, which considerably strengthens the evidence for a third breast cancer susceptibility gene within this genome region.

The respective chromosome 8p region could be narrowed down to 3cM using microsatellite markers. A BAC contig of this region is under construction and some anchor BACs have already been sequenced. Candidate genes or ESTs are tested by mutation analysis. In addition, an electronic Northern blot analysis was performed to obtain differentially expressed genes and these candidates are also included in the mutation testing.

Somatic genetic alterations in breast cancer: Association of breast cancer development and prognosis with genetic alterations

S. Seitz, A. Schwartz, K. Kölbl, K. Poppe, S. Werner in cooperation with P.M. Schlag, M. Dietel and the BCLC

Inter- and intratumoral molecular heterogeneity is one of the characteristics of breast cancer and genetic mechanisms are likely to contribute to it. We have studied loss of heterozygosity (LOH) at specific chromosomal regions in a large panel of breast tumors. The varying incidence of different lesions that we detected indicates intertumoral heterogeneity. We also observed heterogeneity within single tumors, since cases occur in which only some cells within a given tumor have a particular LOH (intratumoral heterogeneity). At present, we are examining the contribution and prognostic relevance of different genetic alterations to the complex process of breast cancer development.

Identification and characterization of genes relevant to breast cancer: YAC and BAC transfer studies and analysis of differentially expressed genes

H. Prokoph, E. Burghardt-Göttges, U.-H. Grasmö-Wendler, S. Seitz, B. Jandrig, R. Frege in cooperation with B. Hinzmann, A. Rosenthal, B. Schlegelberger and B.M. Jockusch

Two distinct chromosomal regions involved in breast cancer were identified by chromosome transfer studies and microsatellite analyses. Our results strongly suggest the existence of tumor suppressor gene(s) in a region distal to TP53 at 17p13.3. Differential display was used to identify differential gene expression between tumor cells and nontumorigenic hybrid cells obtained after transfer of chromosome 17p to tumor cells. More than 150 sequences were cloned and sequenced. One of these sequences, the human profilin 1 gene, a regulator of signal-dependent actin polymerization, has been characterized as a suppressor of the tumorigenic phenotype of breast cancer cells.

Another region for candidate gene(s) of about 10 cM was localized on chromosome 6q23-q25. To identify breast cancer relevant genes, several positional and functional approaches are used in combination: identification of differentially expressed ESTs by electronic- and real Northern blotting and RT-PCR; fine mapping of LOH hotspots; construction of a BAC/PAC contig spanning 1-2 cM; mutation analysis of candidate genes. In addition, functional complementation tests were carried out i.e. BAC/PAC transfer into breast cancer cell lines.

Molecular pathology of solid tumors

K. Kölbl, B. Barthel, L. Estevez-Schwarz, H. Pidde, O.M. Ullrich in cooperation with M. Dietel und P.M. Schlag

We have investigated the patterns of chromosomal and microsatellite instability in human microdissected tumors of the breast, gastro-intestinal and urogenital tract and have correlated these with the prevalence of germ line and somatic mutations in several genes known to be implicated in tumorigenesis (APC, PTEN, CTNNB1, PNF). Integrating these genetic approaches with immunohistological expression analyses has led to the identification of distinct genomic regions on chromosome 17 apparently involved in producing pathomorphologic phenotypes common to a range of different solid tumors. The construction of high resolution STS- and EST-maps has allowed the selection of candidate genes for further mutational screening.

Antibody engineering

B. Micheel, J. Schenk, G. Scharfe in cooperation with U. Heinemann

Antibody technology has been used to produce and modify antibodies against tumor antigens for the diagnosis and therapy of cancer. Experiments using hybridoma technology and phage display are in progress to select monoclonal and recombinant antibodies against epitopes of the BRCA1 gene product. These experiments will increase our knowledge of the structure and function of the BRCA1 gene product and may also provide reagents for the immunohistological diagnosis of breast cancer.

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Clinical and Molecular Genetics of Cardiovascular Diseases

Herbert Schuster

This research group is conducting clinical genetic research into the basic mechanisms of cardiovascular disease. The primary emphasis has been on hypertension and lipid metabolism, although other topics are also being pursued. The approaches have included association studies, linkage analyses, twin studies, and modified sib-pair analyses. Haplotype sharing strategies are planned and will be conducted in the future. The group consists of a genetic field unit which concentrates on recruitment of index patients and their families, recruitment of monozygotic and dizygotic twins, and a laboratory unit which is involved in DNA extraction, genotyping, mutation screening, mutation detection, sequencing and, most recently, functional and positional gene cloning. The major projects are briefly outlined below:

Monogenic diseases

In the summer of 1994, we were informed of an extended family living in northeastern Turkey on the coast of the Black Sea. This family features severe hypertension inherited in an autosomal-dominant fashion and brachydactyly; the two traits exhibit 100% cosegregation. Affected persons have severe hypertension and die of stroke before the age of 50 if untreated. We visited this family and examined over 60 members and mapped the gene to chromosome 12p. To narrow our critical segment, we have identified additional families with this syndrome. A Canadian and an American family, neither of Turkish origin, were located by David Chitayat and Hakan Toka, respectively, and linkage studies in these families have allowed us to narrow our critical segment. We have conducted additional clinical studies and found that all affected individuals have vascular loops involving the posterior-inferior cerebellar artery, which impinges on the ventrolateral medulla. Consistent with the notion that such loops could interfere with baroreceptor function and thereby contribute to hypertension, we have recently shown that patients with this syndrome exhibit orthostatic hypertension and that their baroreflex does not buffer sympathetic tone adequately. In terms of positional cloning, we have identified a candidate gene which we are currently sequencing and are completing our PAC contig.

We are continuing our studies of familial hypercholesterolemia (FH). In cooperation with Eran Leitersdorf and his team in Jerusalem, we have mapped a putative "lipid-lowering" gene in an Arab family living in Israel. In this family, numerous FH-affected persons have normal LDL cholesterol concentrations. By means of several linkage approaches, we have been able to map this modifier gene to chromosome 13q. We have now verified the relevance of this finding by performing a linkage study in dizygotic twin subjects and their parents. We have been able to show that the gene locus on 13q is linked to LDL cholesterol and body mass index in these subjects. The LOD scores from these studies are shown in the figure. We are now expanding these studies to other families in Israel and Germany and are starting positional cloning studies.

Other monogenic projects of the group include the identification of a novel mutation in the elastin gene causing supraaortic stenosis and a mapping study in a child exhibiting holoprosencephaly and renal tubular dysfunction resembling the phenotype in mice with a megalin gene disruption.

Genetic field working unit

A particular strength of the group is its genetic fieldworking and subject recruitment capability. This program was developed by Herbert Schuster and details have been published. In line with the MDC's encouragement of commercial activities, Herbert Schuster has founded INFOGEN GmbH. INFOGEN is a new company specializing in genetic field working and cardiovascular risk assessment. INFOGEN is conducting studies on a large scale throughout Germany. One project concerns establishing the frequency of APO-B mutations as a cause for FH in Germany and determining which APO-B mutations are most commonly involved. This study includes a prospective and a retrospective cohort, each containing over 3000 families. So far, the frequency of APO-B mutations in Germany has been found to be higher than expected. The study will be completed in the year 2000. Another investigation by the group has verified linkage between a chromosome 1q locus and the syndrome of familial-combined hyperlipidemia (FCHL). This finding has been corroborated in Chinese families by Weidong Pei. A concomitant twin investigation by the group has identified an exciting new candidate gene for FCHL.

Twin studies as a strategy to identify quantitative trait loci

Andreas Busjahn and Hans-Dieter Faulhaber have recruited over 200 pairs of monozygotic (MZ) and 120 dizygotic (DZ) normotensive young twins and the parents of the DZ twins. The subjects were carefully phenotyped in terms of blood pressure, blood pressure in response to provocative maneuvers, psychological testing, and serum lipid concentrations. The strategy is to use a standard twin analysis to determine heritability estimates and to distinguish between hereditary and environmental influences. This allows us to perform a standard IBD linkage analysis in the DZ twins and their parents, as well as association studies in the entire twin cohort.

With this approach, we recently identified a series of quantitative trait loci (QTL) relevant to blood pressure regulation. The strongest linkage was found to the IGF-1 gene locus. In collaboration with Margret Hoehe, we have gained new insight into the contribution of the β -2 adrenergic receptor gene. Margret Hoehe's team sequenced the entire β -2 adrenergic receptor gene in our twin cohort and found 15 SNPs, including numerous new mutations. Finally, we have an active cooperation with Per Lund-Johansen's group in Bergen, Norway. From the Bergen Hypertension Study, we have genotyped offspring from two normotensive and hypertensive parents and have been able to associate the Arg16->Gly variant to blood pressure in this cohort.

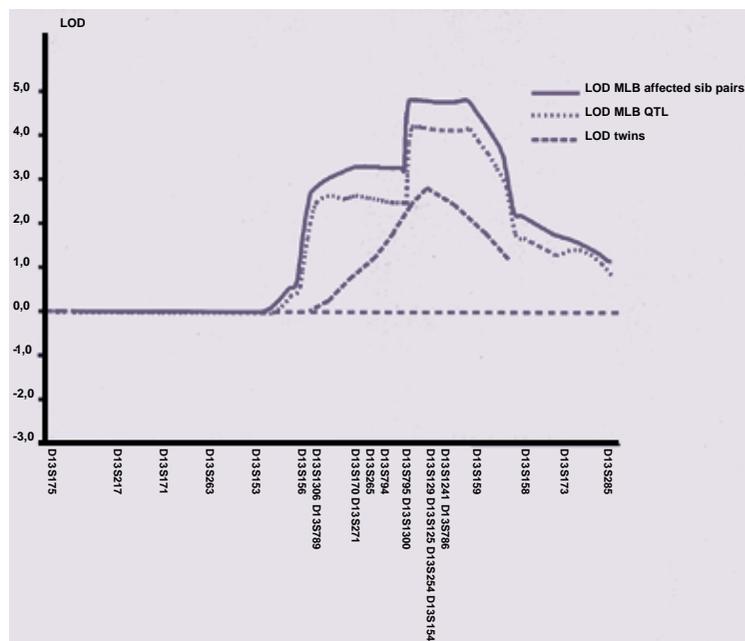
We have used the QTL approach to show that the loci for the long QTc genes, which code for ion channels and their regulators, are all linked to electrocardiogram components. The long QTc syndromes are monogenic diseases associated with sudden cardiac death. Showing relevance of these genes to arrhythmias or risk of arrhythmias in the general population, is the first step in identifying common variants indicating a risk to ventricular arrhythmia. The topic is also highly relevant to the tragic sudden infant death (SID) syndrome. Further studies are in progress to investigate this issue.

Finally, the twin studies have been helpful in identifying a new candidate gene for FCHL. We first looked for linkage between the loci for the peroxisome proliferator-activating protein receptor (PPAR) γ gene and its binding partner, the retinoid X receptor (RXR) gene. The former gene is strongly implicated in the development of obesity. We found that the PPAR γ gene locus is linked to HDL cholesterol and body mass index. Furthermore, the RXR gene locus was strongly linked to triglycerides. Since RXR is located precisely at the chromosome 1q locus linked to FCHL, RXR immediately becomes a very attractive candidate gene for this condition.

New perspectives

Katrin Hoffmann is studying an isolated population in Germany, namely the Sorbs. She has collected 60 families with hypertension and is in the process of performing a total genome scan in cooperation with André Reis. Tom Lindner, who has collected 350 sibpairs with type 2 diabetes from eastern Germany, joins the group after a fellowship with Graeme Bell at the University of Chicago. He is funded to conduct family studies involving a cohort of dialysis patients with type 2 diabetes.

Figure 18: The results of linkage analysis using MLB and MLBQTL in the FH pedigree are shown together with the linkage results for LDL in the DZ twins (p values transformed into LOD scores). In the twins, the peak level of significance was 0.0002, right on marker D13S1241 (Am J Hum Genet 66, 157-166, 2000).



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Mouse Genetics

Carmen Birchmeier

We are using mice for the functional analysis of genes important for development and disease. The molecular genetics of mice is well developed, and homologous recombination combined with embryonal stem cell technology can be used to introduce deletions or insertions into the genome. A further development of the technique, the cre-LoxP technology, now allows us to introduce conditional mutations that are restricted to a particular cell lineage, or subtle alterations like point mutations.

Peripheral nervous system defects in erbB2 mutants

S. Britsch, M. Woldeyesus, D. Riethmacher, E. Sonnenberg-Riethmacher

Neuregulins are EGF-like growth and differentiation factors, which signal via the tyrosine kinase receptors of the ErbB family. We have introduced targeted null-mutations in the erbB2, erbB3 and neuregulin-1 genes. These three mutations cause severe hypoplasia of the primary sympathetic ganglion chain. We have shown that migration of neural crest cells to the mesenchyme lateral of the dorsal aorta, where they differentiate into sympathetic neurons, depends on neuregulin-1 and its receptors. A close association between neuregulin-1 expression and the migratory path and the target site of sympathogenic neural crest cells has been observed. Moreover, these mice show severe defects in the development of Schwann cell precursors and their cardiac system.

ErbB2^{-/-} mice die at midgestation due to heart malformation. We have been able to genetically rescue their heart development by myocardial expression of erbB2 cDNA. In rescued erbB2 mutants, Schwann cells are lacking. Motoneurons form and can project to muscle, but nerves are poorly fasciculated and disorganized. Although neuromuscular junctions form, there is a severe loss of cervical and lumbar motoneurons, but not of thoracic ones. These results define the roles of Schwann cells during motoneuron and synapse development and show that Schwann cells generate important survival factors for distinct motoneuron populations. Our analysis provides genetic evidence that the major developmental role of ErbB2 is to provide a co-receptor function for the neuregulin receptors ErbB4 and ErbB3.

A role for erbB2 in myelination

A. Garratt

Neuregulin-1 provides an important axonally-derived signal for survival and growth of developing Schwann cells, which is transmitted by ErbB2/ErbB3 receptor tyrosine kinases. Null-mutations of the neuregulin-1, erbB2 and erbB3 mouse genes cause severe deficits in early

Schwann cell development, and the mutant mice do not develop beyond birth. We employed Cre-loxP technology to introduce erbB2 mutations late in Schwann cell development, using a Krox20-cre allele. Cre-mediated erbB2 ablation occurs perinatally in peripheral nerves. The mutant mice exhibit a widespread peripheral neuropathy characterized by abnormally thin myelin sheaths, containing fewer myelin wraps. Thus, the Neuregulin signaling system functions during multiple stages of Schwann cell development and is essential for proper myelination. The thickness of the myelin sheath is determined by the axon diameter, and we suggest that trophic signals provided by the nerve determine the number of times a Schwann cell wraps an axon.

The cryptic gene is essential for correct establishment of the left-right axis

U. Gaio, A. Garratt, T. Müller, C. Özcelik, W. Lankes, M. Strehle

During vertebrate embryogenesis, a left-right axis is established. The heart, associated vessels and inner organs adopt asymmetric spatial arrangements and morphologies. Thus, the apex of the heart points to the left side of the body, the liver is located on the right side, stomach and spleen on the left, right and left lung differ in lobation, and the gut is asymmetrically curled. We have generated a mutant allele of cryptic, an EGF-CFC gene in the mouse. Homozygous cryptic mutants develop to birth and die during the first week due to complex cardiac malformations that include malpositioning of the great arteries, and ventricular and atrial septal defects. A variety of laterality defects are observed, such as randomised heart looping, right isomerism of the lung, and splenic hypoplasia. This phenotype is reminiscent of the asplenic syndrome in humans that is typically associated with laterality defects and malpositioning of the great arteries.

Lbx1, c-met and the control of cell migration of muscle precursor cells

H. Brohmann

Muscle of the extremities is generated by migrating myogenic precursor cells. These precursors delaminate from the lateral edge of the dermomyotome and form distinct streams that migrate over large distances, using characteristic paths. We are characterising the genetic hierarchy that controls the migration of this lineage. We have previously shown that the c-met tyrosine kinase receptor and its ligand, SF/HGF, are essential for the delamination of cells. Moreover, SF/HGF is expressed along the entire migratory route of muscle precursor cells, indicating that this signaling system plays a role also during the migration process. Indeed, we are currently analysing mice with reduced c-met signaling capacity, which show abnormal limb muscle development and abnormal migration of precursor cells. The homeobox gene *Lbx1* is expressed in migrating, but not in other types of muscle precursor cells. We have used gene targeting to analyse the function of *Lbx1* in the mouse. Myogenic precursor cells delaminate from the dermomyotome in *Lbx1* mutants, but migrate in an aberrant manner, and do not reach the dorsal limb field. In the ventral limb, precursors are present but distributed abnormally. As a

consequence, at birth some muscles in the forelimbs are completely lacking (extensor muscles) or reduced in size (flexor muscles).

Selected Publications

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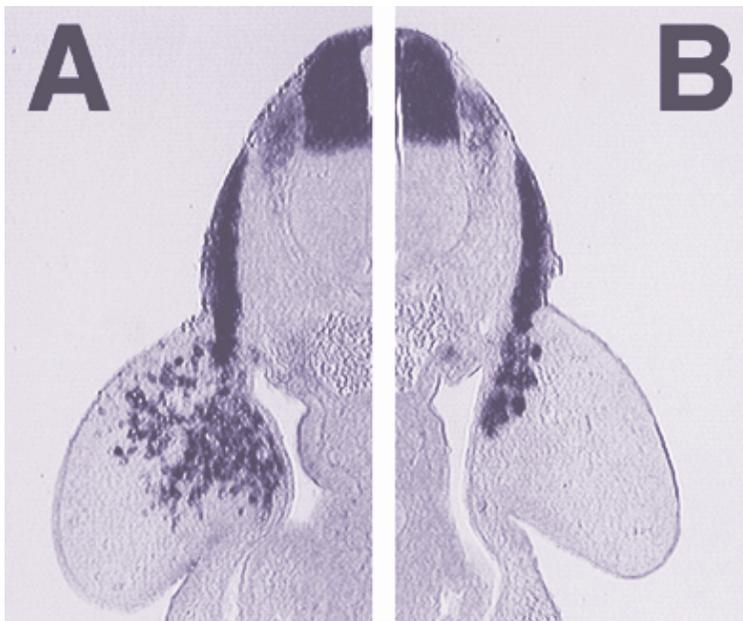


Figure 19: Aberrant migration of myogenic precursor cells in *Lbx1* mutant embryos. Myogenic precursor cells in control (A), and *Lbx1*^{-/-} (B) embryos at E9.75 were visualized by *in situ* hybridization using a *Pax3* specific probe. Myogenic precursor cells detach from the dermomyotome in *Lbx1* mutants, but do not migrate appropriately to the limb bud. In control embryos, the precursor cells have reached the limb bud at this stage.

Developmental Genetics

Andreas Schedl

Development and disease are two closely linked processes and many disorders can be explained by abnormal cellular differentiation. Hence, it is essential to understand the molecular basis of development and differentiation, if we want to develop therapeutic interventions for human diseases. Our group is particularly interested in developmental aspects of the urogenital and cardiovascular system. Using transgenic strategies in mice we are analysing the molecular function of several transcription factors and cell surface receptors, developing model systems for human diseases and trying to understand the cellular and pathophysiological processes leading to the diseased phenotype in mouse and man.

WT1 in development and disease

WT1 is a zinc finger protein, which has been shown to be mutated in a percentage of Wilms tumours, an embryonic kidney tumour arising from undifferentiated mesenchymal cells. Dominant mutations have also been found in patients suffering from the Frasier and Denys-Drash Syndromes, both of which are characterised by abnormal gonadal development and defects in glomerular function leading to end-stage renal failure early on in life. WT1 expression shows a very distinct expression pattern throughout kidney development, with low levels in the undifferentiated blastema, slightly higher levels in the developing nephron and the highest levels within the podocyte layer, the filtrating cell type in the kidney. To investigate the function and regulation of the WT1 gene at various stages of development, we have generated transgenic mice carrying the human WT1 locus. Using a lacZ reporter gene inserted into a YAC construct, we have demonstrated that WT1 is expressed in the early proepicardium, epicardium and subepicardial mesenchymal cells (SEMC). Lack of WT1 leads to severe defects in the epicardial layer and a concomitant absence of SEMCs, which explains the pericardial bleeding and subsequent embryonic death observed in Wt1 null embryos. A human-derived WT1 YAC construct is able to completely rescue heart defects, but only partially rescues defects in the urogenital system. Our analysis of the observed dysplastic kidneys demonstrates a continuous requirement for WT1 during nephrogenesis, in particular, in the formation of mature glomeruli. Furthermore, we have demonstrated that the development of adrenal glands is also severely affected in partially rescued embryos. Our data support a variety of new functions for WT1 and suggest a general requirement for this protein in the formation of organs derived from the intermediate mesoderm. Using transgenic mice, we are presently mimicking several other diseases caused by WT1, including the Frasier and Denys-Drash syndromes. These analyses will allow us to gain additional insight into the molecular function of WT1, the etiology of WT1 diseases and, hopefully, allow us to develop therapeutic interventions.

WT1, SOX9 and the determination of sex

Sex determination is a fascinating process in which an undifferentiated gonad develops either into a testis or ovary depending on the presence or absence of a single gene, the SRY gene. Expression of SRY initiates a molecular cascade, which eventually results in the expression of an important male specific signalling molecule, the Mullerian inhibiting substance (MIS). The precise factors required for the activation of MIS are still unclear, but a current model suggests that a combination of transcription factors WT1, SOX9 and SF1 may synergistically activate the MIS promoter. We are presently testing this model by specifically adding or removing some of these factors in an *in vivo* situation. In addition to being involved in gonad specification, SOX9 is also important for normal differentiation of bones and mutations have been found in the syndrome campomelic dysplasia (CD). Patients present with either heterozygous mutations in the SOX9 gene or chromosome rearrangements mapping at least 50 kb upstream of SOX9. Whereas mutations within the coding region of SOX9 cause haploinsufficiency, the effects of translocations 5' to SOX9 are unclear. To test whether these rearrangements also cause haploinsufficiency by altering spatial and temporal expression of SOX9, we have generated mice transgenic for human SOX9-lacZ yeast artificial chromosomes containing variable amounts of DNA sequences upstream of SOX9. We have shown that elements necessary for SOX9 expression during skeletal development are highly conserved between mouse and human and found that a rearrangement upstream of SOX9, similar to that observed in CD patients, leads to a substantial reduction in SOX9 expression, particularly in chondrogenic tissues. These data demonstrate that important regulatory elements are scattered over a large region upstream of SOX9 and explain how particular aspects of the CD phenotype are caused by chromosomal rearrangements 5' to SOX9.

Selected Publications

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Patent Application

Shuttle vectors for BACs and YACs

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Lipids and Experimental Gene Therapy

Thomas E. Willnow

The low density lipoprotein (LDL) receptor is a 150 kDa endocytic receptor that mediates 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), a 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 new receptors have been identified that are structurally related to the LDL receptor and form members of the LDL receptor superfamily (Figure 18). The significance of these receptors for regulation of systemic and cellular lipid metabolism is unknown. We are using gene targeting and somatic cell gene transfer approaches to generate mouse models with deficiencies in LDL receptor-related receptors and to study the consequence of such receptor defects *in vivo*.

The LDL receptor-related protein (LRP) is a 600 kDa cell surface receptor and a member of the LDL receptor gene family. Because LRP is highly expressed in hepatocytes, it has been speculated that the receptor may play a role in the hepatic uptake of dietary lipoproteins. Dietary lipoproteins are produced by the intestine and transport lipids and lipid-soluble vitamins absorbed from the diet. These particles are cleared from the circulation into the liver via hepatic lipoprotein receptors. The LDL receptor mediates hepatic uptake of dietary lipoproteins; however, it is not the only receptor to do so, because systemic clearance of dietary lipoproteins is normal in patients with FH. To test the contribution of the LRP to the hepatic uptake of dietary lipoproteins, we analyzed mice functionally deficient in the LDL receptor, LRP or both receptors. Using these animal models, we have been able to confirm that the clearance of dietary lipids proceeds via a dual lipoprotein receptor system, consisting of the LDL receptor and the LRP.

Megalin is another member of the LDL receptor gene family. This receptor is predominantly expressed on the epithelial cells of the proximal tubules in the kidney. Experimental evidence suggests that the receptor may be involved in the uptake of macromolecules from the glomerular filtrate. To test this hypothesis and to identify its endogenous ligands, we generated mice genetically deficient in 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 D₃, and demonstrated that the receptor mediates the tubular retrieval of vitamin/DBP complexes filtered through the glomerulus. This receptor-mediated uptake is required to prevent the loss of vitamin D₃ metabolites by glomerular filtration.

Furthermore, it delivers 25-(OH) vitamin D₃ to tubular epithelial cells for conversion into 1, 25-(OH)₂ vitamin D₃, the active form of the vitamin and a potent regulator of systemic calcium and bone metabolism. Urinary excretion of 25-(OH) vitamin D₃ in megalin^{-/-} mice results in vitamin D deficiency and impaired bone formation. 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 D₃ metabolites.

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 physiology and pathophysiology of lipid metabolism.

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Herz, J., Willnow, T.E., and Farese, Jr. R.V. (1997) Cholesterol, hedgehog and embryogenesis. *Nature Genet.* 15, 123-124.

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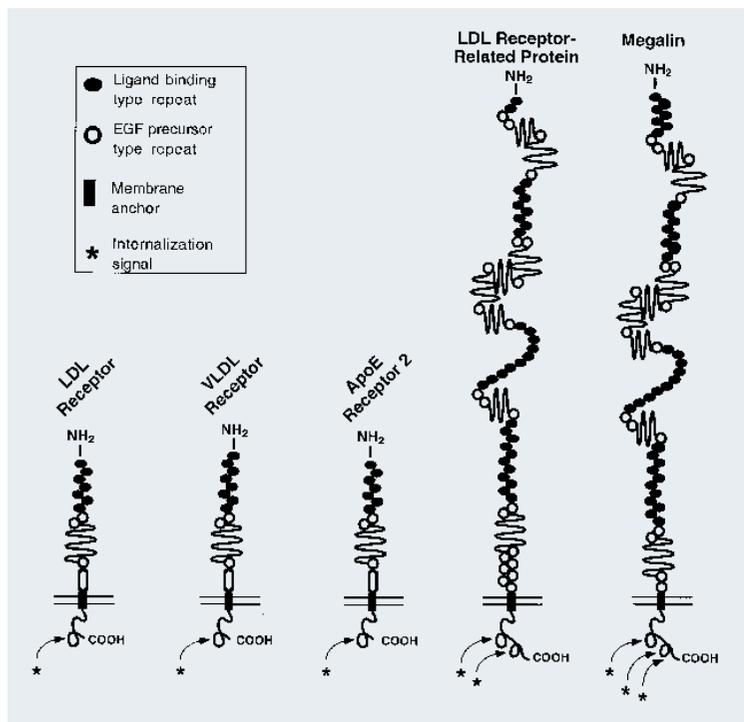


Figure 20: Structural organization of mammalian receptors of the LDL receptor gene family. Structural elements common to all members of the LDL receptor superfamily are depicted. These include (i) ligand-binding type repeats (filled circles) and epidermal growth factor (EGF) precursor type repeats (open circles), (ii) a single transmembrane domain (filled square) and (iii) internalization signals (asterisk) in the cytoplasmic tail that direct the receptors into coated pits.

We aim to study the genetic epidemiology of normal and aberrant lipid metabolism in man. The contribution of genotype, gene expression type and conditioning environmental factors will be addressed in a systematic manner, making use of metabolic models of the lipoprotein system.

Selected Publications

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Interactions of Biopolymers in Solution

Joachim Behlke

Our group is engaged in the analysis of the structure of proteins and nucleic acids in solution and their interactions using analytical ultracentrifugation methods. Special programs have been developed that allow us to determine the gross conformation of polymers, self- and hetero-association as well as parameters of thermodynamic nonideality. The substances investigated are of medical and biotechnological relevance and the data obtained may help us understand possible regulatory mechanisms of transcription or protein folding and metabolic pathways within the cell.

Gross conformation of peptides

To obtain estimates of the possible shape of angiotensin peptides which bind to the AT1 receptor (seven-transmembrane-helix G-protein-coupled complex), we have analysed the gross conformation of these peptides using measurements of hydrodynamic mobility and theoretical calculations. The most probable, extended structure of angiotensin 2, about 3 nm in length with a kink, seems to penetrate approximately 2 nm into the AT1 receptor where it binds to specific amino acids and induces the complex reaction.

Regulation of oligomeric protein structures and their consequences

Collaborations with E.-C. Müller, A. Otto, MDC, and T. Kriegel, TU Dresden (hexokinase), P. Tavares, Inst. Pasteur, Paris (portal protein SPP1) and S. Brantl and K. Steinmetzer, Univ. Jena (CopR)

Homodimeric hexokinase 2 from *Saccharomyces cerevisiae* has one phosphorylation site at Ser 14. This modification is triggered *in vivo* by glucose exhaustion. We have shown that, following site-directed mutagenesis (Ser 14 exchange by Glu) or phosphorylation, the dimeric enzyme dissociates completely into monomers. We assume that the *in vivo* phosphorylation at Ser 14, as transiently occurs in low glucose states, may be a mechanism to improve glucose utilization at low levels and / or that nuclear localization of the monomer may be involved in signal transduction whereby glucose causes catabolite repression.

Bacteriophage SPP1 portal protein is a large cyclic homo-oligomer composed of 13 subunits. It is stable in the presence of 10-50 mM MgCl₂. Decreasing electrolyte concentration leads to a reversible dissociation into monomers which are partially unfolded. The reassociation of monomers into the 13-mers requires a chaperone-independent folding of monomers in the presence of Mg⁺⁺.

CopR binds as a dimer with high affinity to two consecutive major grooves (site I and site II) of the DNA (K_D = 0.4 nM). The complex

formation is a coupled process and its analysis requires knowledge of the preceding CopR dimerization which has a dissociation constant of 1.4 μM. Since the cellular concentration of CopR is about 20-fold higher than the dimerization constant we can assume that CopR binds *in vivo* as a preformed dimer.

Recognition of peptide sequences at the interface of homodimeric proteins

Collaboration with W. Höhne, Humboldt-Univ., Berlin

To map the putative dimerization site in the capsid protein p24 (HIV-1) a set of overlapping peptides spanning the p24 sequence was synthesized and tested for the ability to modify the monomer-dimer equilibrium. Most of the candidates were inactive. However, one peptide was found to compete with the monomers in the dimerization reaction. This sequence, therefore, may be part of the contact region between two monomers.

Nucleic-acid protein interaction

Collaboration with A. Rich, MIT, Cambridge, MA, and H. Oschkinat, Inst. of Molecular Pharmacology, Berlin

The Z domain of the human RNA editing enzyme double-stranded RNA deaminase I (ADAR1) binds to left-handed Z-DNA with high affinity (K_D = 30 nM). Using sedimentation equilibrium techniques and CD spectroscopy, we found that two Z domains bind to one d(CG)₃T₄(CG)₃ hairpin which contains a stem of six base pairs in the Z-DNA conformation. We suggest that short segments (6 bp) of the Z-DNA within a gene are able to recruit two ADAR1 enzymes to that particular site.

Nucleation

Nucleation as a pre-requisite for the crystallization of proteins can be considered as a special case of self-association. Using sedimentation velocity experiments performed under crystallization conditions, we were able to detect oligomers of 15-20 protein molecules. These complexes or nuclei can grow spontaneously to crystals in supersaturated solution. Crystallization conditions are often far from the pI, where proteins are either polyanions or polycations. The high net charge, as reflected by nonideality data (second virial coefficient), can prevent oligomerization. By addition of neutral salts the charges are screened resulting in a reduction in repulsion between the protein molecules and the possibility of forming associates. The conditions necessary for protein crystallization can be derived from the value of the virial coefficient or the ratio of the excluded volume and the charge-dependent part of this parameter.

Selected Publications

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Folding and Misfolding of Proteins

Gregor Damaschun

The creation of proteins in living cells consists of two main processes: biosynthesis of the polypeptide chain and its folding into the native, three-dimensional structure with biological function. The first process has been thoroughly studied, while the second process is less well known. We have learnt in recent years that the protein-folding process is not always flawless within the cell and this can have pathological consequences. Thus, a number of human diseases are related to the deposition of protein fibrils causing tissue damage and degeneration. Amyloid fibrils develop from abnormal, misfolded conformational states of different normally soluble proteins forming ordered aggregates. The reasons for misfolding are unknown. Therefore, there are no causal treatments for these diseases. The group "Physics of Biopolymers" is engaged in studies of the folding pathways of proteins to understand the causes of misfolding. The main experimental methods used in these studies include solution X-ray scattering (SOXS), dynamic light scattering (DLS) and optical spectroscopy, including kinetic techniques. Methods of statistical physics of chain molecules have been applied to modelling the experimental data.

Polymorphism of proteins

Textbooks state that the structure of a protein is determined by its amino acid sequence. However, we have been able to show experimentally that this so-called second genetic code is not unambiguous. The three-dimensional structure of a protein is determined not only by the amino acid sequence but also by the environment of the protein molecules and is influenced by interactions between structural intermediates on the folding pathway. Therefore, many proteins can adopt differently folded three-dimensional structures and only one of these structures is functionally active. For yeast phosphoglycerate kinase (PGK), we observed in addition to the native structure two further, different conformations. The starting point for the formation of these misfolded conformations is the acid-unfolded state. At low pH values, PGK has the conformation of an expanded random walk. If the molecule is transferred to a hydrophobic environment with a low dielectric constant, the entire molecule forms α -helix. On the other hand, anion-induced partial refolding of the acid-unfolded state leads to the formation of amyloid-like fibrils. Half the amino acids have the conformation of a cross- β -helix which is typical of all amyloids.

Folding pathways and kinetics

The formation of amyloids starts from non-natively folded monomeric intermediates. The monomers aggregate forming successively dimers, tetramers and octamers. More and more cross- β -structure develops during this aggregation process. The kinetics of aggregation is strongly dependent on protein concentration. At room temperature, this process may take several hours. Subsequently, the octamers grow in one direction only and form fibrils. The growth of the fibrils, i.e. their time-dependent elongation, may take some months. Our results indicate that inhibitors of cross- β -structure formation can be effective only during the early phases of amyloidosis. The slow kinetics are typical of misfolding of proteins into amyloids. *In vivo*, the progression of these processes is in some cases even slower than in our *in vitro* experiments. By contrast, the folding of a protein into its native structure is a fast process. Typical times for folding vary from milliseconds to minutes. One central problem in protein folding is the question, whether chain segments with a periodic secondary structure develop in a first step, then form in a second step the compact globule through diffusion (framework model), or whether the chain initially collapses, driven by hydrophobic interactions, with concurrent or subsequent formation of segments with periodic secondary structure (hydrophobic collapse model). We have been able to show experimentally that both models are not general alternatives. There are proteins folding mainly according to the mechanism of the framework model (e.g., bovine RNase A) as well as folding according to the hydrophobic collapse model (e.g., bovine α -lactalbumin). Further studies are necessary to address the open question: which of these folding scenarios is more prone to the misfoldings that lead to amyloids? Up to now, a search for common properties of amyloid-forming proteins has been unsuccessful.

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Structure of the Group

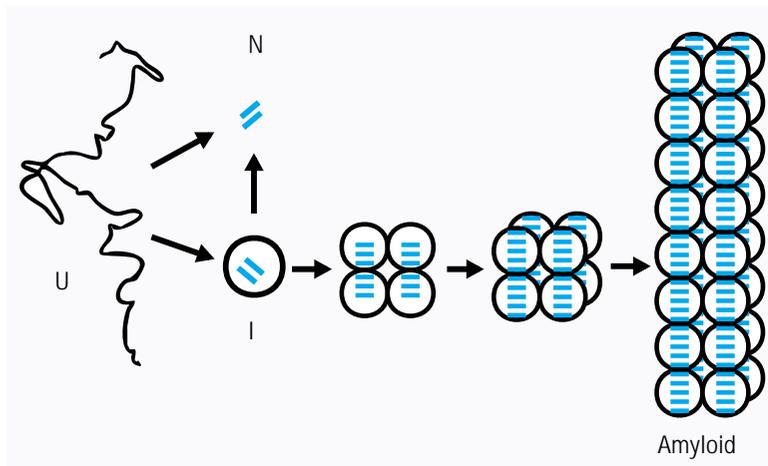
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Figure 22: Formation of amyloid fibrils by misfolding of proteins. The blue bars represent cross- β -structures of the polypeptide chain. U- unfolded state in an acidic environment, N- native state, I- folding intermediate.



Protein Folding and Misfolding

Heinz Fabian

Understanding the mechanism of protein folding is of considerable clinical importance since a number of diseases, such as Alzheimer's disease and spongiform encephalopathies, are pathological consequences of misfolding. A characteristic feature of various medical disorders is the self-assembly of β -sheet domains resulting in the formation of pathogenic protein aggregates (amyloid fibrils). A growing body of data suggests that partially folded intermediates are precursors of such aggregates. Fourier transform infrared (FTIR) spectroscopy is particularly good at detecting the presence of and changes in β -sheet structures. Moreover, the FTIR approach now allows the investigation of *in vitro* protein unfolding/folding events in the millisecond to minute time scale and we have applied infrared spectroscopic techniques to investigate folding and misfolding processes in peptides and proteins.

Peptides and proteins capable of forming amyloid fibrils *in vitro*

in collaboration with E.-G. Krause (Research Institute for Molecular Pharmacology, Berlin)

The principles behind β -sheet formation are not well understood due to difficulties in the development of simple model systems. The design of β -sheet peptides is complicated by their limited solubility in water and due to the nature of their folding, which is dictated by long range interactions. We have described de novo β -sheet peptides which self-assemble into fibrillar structures. The influence of peptide length, concentration, and D-amino acid substitution on the ability to form amyloid fibrils has been analysed. Our results suggest that amyloid formation is not restricted to very few peptide sequences associated with disease states. Conformational studies of synthetic analogs of Alzheimer β A4 peptides have revealed that the central hydrophobic region plays a key role in the conformational switch of the peptide.

in collaboration with G. Damaschun (MDC)

Although unrelated to proteins involved in known amyloid diseases, phosphoglycerate kinase is capable of forming amyloid fibrils under certain conditions. The ability to design conditions under which fibril formation can be observed with otherwise soluble proteins offers the opportunity to investigate the molecular mechanism of the underlying process. In the case of yeast phosphoglycerate kinase, a rapidly formed and partially folded monomeric intermediate involved in the aggregation process has been detected.

Folding of the enzyme ribonuclease T1

in collaboration with D. Naumann (Robert Koch-Institute, Berlin)

Folding of the model protein ribonuclease T1 is known to be complex, involving several fast and slow phases. Our time-resolved infrared studies have provided new insights into the structural events accompanying the folding of ribonuclease T1. In particular, an extremely slow folding process has

been observed, which was correlated with restricted structural changes due to an isomerization of the proline-39 bond in the protein.

Unfolding and folding of the lambda Cro repressor protein

in collaboration with V.V. Rogov (Institute of Protein Research, Russia), K. Gast (MDC) and H.H. Mantsch (Institute for Biodiagnostics, Canada)

The λ -Cro repressor is one of the proteins which can be used as a model system to study the interplay between changes in secondary structure and the state of association upon unfolding and refolding. In the active state of the Cro repressor protein, two monomeric units form a dimer by aligning the C-termini of each monomer, allowing the formation of an antiparallel β -ribbon across the dimer. The N-terminal parts form small globular subdomains that consist of three α -helices and a short N-terminal β -strand connected to the β -ribbon. Conventional FTIR and dynamic light scattering experiments have shown that the first thermal transition of a variant of Cro, which contains a disulfide cross-link between the protein subunits in the dimer, only involves unfolding of the three α -helices and the short N-terminal β -strand. The intermediate state has a well structured intermolecular β -sheet domain still formed by the C-terminal parts of each polypeptide chain and associates into a tetrameric structure. This stable intermediate unfolds during a second transition at higher temperatures, a process that is also accompanied by dissociation of the tetramers. Our kinetic infrared studies have revealed that oligomerization of the covalently cross-linked protein strongly decelerates its folding. Analysis of structural changes applying 2D-IR correlation spectroscopy, a novel experimental approach, has provided fundamental insights into sequential events in the formation and also unfolding of the stable intermediate of the Cro protein.

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

Udo Heinemann

We rely on macromolecular crystallography to study structural aspects of proteins and nucleic acids. The crystal structures of these molecules serve to explain their function in biological processes, conformational stability and folding. General areas of interest include nucleic acids and nucleic-acid binding proteins, electron transport in cytochrome P450 systems and the structural determinants of the stability and folding of globular proteins. Y.A. Muller is engaged in studies of hormone transport by the human sex hormone-binding globulin and of tissue factor. Many of these projects involve collaborations with scientists from Berlin and elsewhere. In addition, there is a growing number of in-house collaborations focussing, for example, on Wnt signal transduction involving β -catenin and conductin, inhibitors of the transcription factor NF- κ B and G-protein coupled receptors. In the newly developing field of structural genomics, we have helped create a Berlin-based research project, the Protein Structure Factory (PSF). Here, the aim is to set up a local infrastructure for the semi-automated, low-cost, high throughput structure analysis of proteins. The PSF contributes to a world-wide effort to determine the structures of a representative set of protein domains that will greatly facilitate future protein modelling and drug design studies.

Nucleic acids and interacting proteins

H. Delbrück, A. Diehl, O. Gaiser, H. Lauble, U. Müller, Y. Roske, E. Werner

The sequence-specific recognition of nucleic-acid molecules by proteins and other ligands is thought to be mediated by local structural features of the nucleic acid. We have determined the crystal structures of several synthetic RNA molecules in an effort to identify the determinants of specific protein binding. A chimeric DNA-RNA hybrid, that corresponds to the RNA-DNA junction formed during minus-strand synthesis in the course of reverse transcription of the HIV-1 genome and carries specific cleavage sites of the reverse transcriptase-associated ribonuclease H, has been shown to adopt the standard A-type conformation. The cleavage specificity of the ribonuclease H has been suggested to be associated with a structural perturbation of the sugar-phosphate backbone at the main cleavage site. In another study, the crystal structure of the acceptor stem helix of tRNA^{Ala} was determined at atomic resolution from pseudo-merohedrally twinned crystals. Here we have been able to show that the G·U wobble base pair known to be crucial for tRNA recognition by the cognate tRNA synthetase is hydrated in a characteristic way and embedded in the unperturbed, standard A-form RNA. Significant progress has been made in the structure analysis of several nucleic-acid binding proteins. The C-terminal domain of the transcription factor KorB was determined at high resolution and shown to adopt a SH3-like fold responsible for KorB dimer formation. For the complex formed between the C-terminal domain of translation initiation factor IF2 and initiator tRNA, crystallization and X-ray diffraction conditions will have been optimized to allow completion of the structure analysis in the near future.

Electron transport in cytochrome P450 systems

J.J. Müller

In vertebrates, enzymes of the cytochrome P450 family catalyse a variety of chemical reactions, including steroid hormone

biosynthesis. They receive electrons from a [2Fe-2S] ferredoxin which, in turn, accepts electrons from an NADPH reductase. We have determined the crystal structure of adrenodoxin, the ferredoxin from the bovine adrenal gland mitochondrial matrix, at 1.85 Å resolution (Figure 21). In spite of the low-level sequence similarity, adrenodoxin bears close structural similarity to the well known class of plant-type [2Fe-2S] ferredoxins and appears to share with these proteins a common mode of docking to the cognate reductase and predicted electron transfer pathway. Very recently, we have been able to solve the crystal structure of the chemically cross-linked complex of adrenodoxin with adrenodoxin reductase which will allow us to model electron transfer between these proteins with some confidence. The crystal structures of adrenodoxin and its complex with the adrenodoxin reductase serve to explain a large body of biochemical and mutational data.

Structural basis of protein stability and folding

J. Ay, A.M. Babu, H. Delbrück, U. Müller

Selected aspects of protein folding and thermodynamic stability can be related to the native three-dimensional protein structure as determined by X-ray crystallography. Over the last two years, we have studied three different model protein families in this respect. Biochemical and crystallographic analyses of 1,3-1,4- β -glucanases have shown that the jellyroll fold of these proteins resists various circular permutations of the protein sequence and, in the case of the engineered protein GluXyn-1, even transplantation of the autonomous folding unit of a xylanase into a surface loop of the protein. These studies have been expanded using the protein thiol/disulfide oxidoreductase DsbA, where we have demonstrated by crystal structure analysis that moving the polypeptide chain termini from the thioredoxin-like domain into the α -helical domain by circular permutation of the sequence has little effect on the three-dimensional protein structure. Finally, we are currently investigating pairs of bacterial cold-shock proteins of closely similar sequence but drastically different conformational

stability. By determining the structure of the cold-shock protein, *Bc-Csp*, at atomic resolution we have shown that its gain of more than 20 °C in thermal stability over a mesophilic homolog is entirely due to electrostatic interactions of two exposed surface residues. These findings open exciting new possibilities for protein engineering aimed at creating proteins of predetermined stability.

Plasma sex steroid transport by SHBG

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

Human sex hormone-binding globulin (SHBG) is the major sex steroid carrier in blood. In biological fluids, SHBG exists as a homodimer and each monomer comprises two G-modules. These modules are about 200 residues long and occur in a variety of proteins such as extracellular matrix proteins, proteins involved in blood coagulation and ligands of receptor tyrosine kinases. We recently solved the crystal structure of the amino-terminal G domain of SHBG in a complex with 5 α -dihydrotestosterone and characterized both the architecture of the steroid binding site and the quaternary structure of the dimer. We have shown that G domains have jellyroll topology and are structurally related to pentraxin. In each SHBG monomer, the steroid intercalates into a hydrophobic pocket within the β -sheet sandwich. The steroid and a 20 Å distant calcium ion are not located at the dimer interface. Instead, two separate steroid binding pockets and calcium binding sites exist per dimer. The structure shows why SHBG is able to bind a variety of synthetic steroids used, for example, as contraceptives. Future research will focus on the crystallisation of SHBG with various natural and synthetic steroids.

Tissue factor, a member of the cytokine receptor superfamily

K. Fälber, Y.A. Muller

Tissue factor (TF), the obligate cofactor for coagulation factor VIIa (FVIIa) is a member of the cytokine receptor family. Like growth hormone receptor, TF is an integral membrane glycoprotein with a 219 residues long ectodomain, a transmembrane segment (23 residues) and a cytoplasmic domain (21 residues). Crystal structures of the ectodomain of TF and other members of the cytokine receptor family have been reported. However, until now, nobody has succeeded in crystallizing an integral receptor, thus leaving many questions related to the signal transduction mechanism unanswered. Our crystallization trials on solubilised intact TF, with or without monoclonal antibodies, have yielded crystals but, so far, their quality has not led to a successful structure analysis.

Figure 23: Crystal structure of the truncated bovine adrenodoxin Adx(4-108) (A. Müller et al., 1998). α -Helices and β -sheets are shown as blue spirals and grey arrows, respectively, and the atoms belonging to the [2Fe-2S] cluster are shown explicitly. The core domain of the protein (right) is preserved in plant-type [2Fe-2S] ferredoxins, whereas the interaction domain responsible for docking to adrenodoxin reductase or cytochrome P450 (left) is different in the two subclasses of vertebrate and plant-type proteins.



Selected Publications

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Role of Protein Dynamics in Enzyme Function

Christiane Jung

The dynamic behaviour of protein structures and their 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 cytochromes P450 are involved in several metabolic processes in animals and humans, such as 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, nitric oxide. Both enzymes have a very similar heme iron coordination sphere and analogous intermediate steps in their reaction cycles, although their biological function, secondary structure and interaction with redox partners are very different. Uncovering the fundamental structural requirements for this different behaviour may contribute to a better understanding of the reaction mechanisms of heme protein enzymes and to the development of new strategies for the design of enzyme inhibitors which is of great medical importance.

In the last two years we have focussed on the structure analysis of cytochromes P450 from various sources and of inducible mouse NO synthase in different states of the reaction cycle using Fourier transform infrared (FTIR) spectroscopy. Bacterial cytochrome P450cam has also been studied in cooperation with other groups using NMR, EPR and Mössbauer spectroscopy.

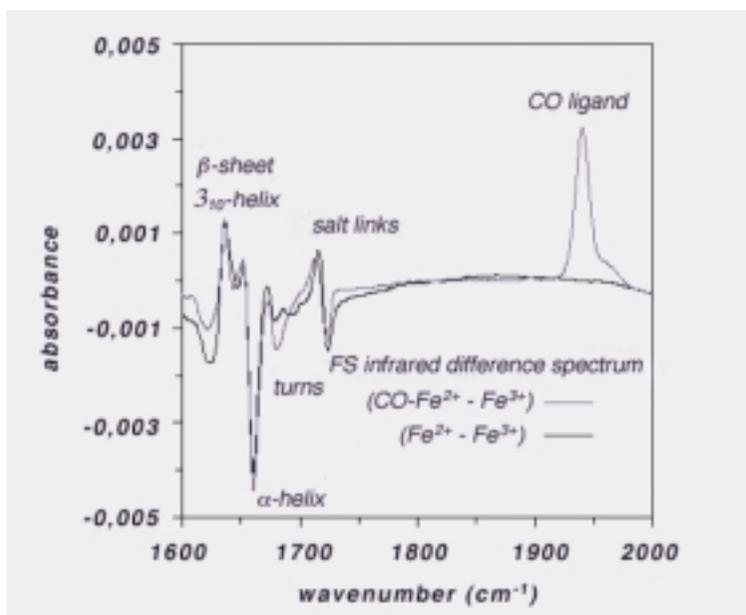
Structural changes implicated in electron transfer in cytochrome P450

Within the last few years we have established the laser flash-photolysis technique coupled with time-resolved FTIR spectroscopy. This technique allows study of the photoinduced processes like heme iron ligand binding or photoreduction of heme iron. We have found that reduction of the heme iron, as well as the binding of iron ligands, induce changes in the secondary structure of the protein and in intramolecular salt links between the heme propionic acids and the protein (see figure). It has been established that formation or changes in salt links also play a significant role in the intermolecular electron transfer from redox partners (iron-sulfur proteins) to cytochrome P450 and infrared spectroscopy is able to detect these changes. In cooperation with F.W. Scheller and his group, University of Potsdam, we have shown that bacterial cytochrome P450cam can also be reduced electrochemically with a high electron transfer rate, similar to that seen physiologically, if the electrode is modified by a negatively charged clay which obviously mimics the electrostatics of the natural redox partner, putidaredoxin. Electron transfer rates between putidaredoxin and cytochrome P450cam have been determined in collaboration with G. Simonneaux and his group, University Rennes I, France, using the proton NMR technique.

Unstable reaction intermediates of cytochrome P450

The reaction cycle of thiolate heme proteins proceeds via an unstable intermediate, called compound I, or [Fe-O]-species. The electronic structure of this intermediate has not been characterized so far but it is thought to be similar to the corresponding intermediates of peroxidases. In cooperation with A.X. Trautwein and his group, Medical University of Lübeck, we have stabilized this intermediate in freeze-quench experiments and characterized it by Mössbauer and EPR spectroscopy. It turns out that the heme iron is in the Fe(IV) state, but the electronic structure of the whole species differs from that of peroxidases.

Figure 24: Fourier transform infrared absorption difference spectrum induced by tris-bipyridyl-Ru(II)-complex-mediated photoreduction of IR-camphor-bound cytochrome P450cam in the presence and absence of carbon monoxide (100 mM deuterated potassium phosphate buffer, pH 7, 1 mM Ru(II)(bpy)₃Cl₂, 10 mM EDTA, 10 % (v/v) glycerol-d₃, 13 mM IR-camphor; 23 μm pathlength, 532 nm excitation by Nd-YAG laser)



Active site structure of iNO synthase

In cooperation with D.K. Ghosh, Duke University Medical Center, Durham, USA, we have analysed the effect of arginine and tetrahydrobiopterin on the active site structure of inducible NO synthase (oxygenase domain) from mouse using the FTIR spectroscopy over the broad temperature range from 20 K to 298 K. The CO stretch mode of the heme iron CO ligand has been used as a spectroscopic probe. We have shown that tetrahydrobiopterin has no significant effect on the active site structure. In contrast, arginine forms a hydrogen bond to the CO ligand and makes the active site more rigid. This suggests that a hydrogen bond may also exist in the physiologically relevant dioxygen complex which would have significant consequences for the reaction mechanism.

Selected Publications

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Mouro, C., Bondon, A., Jung, C., Hui Bon Hoa, G., De Certaines, J.D., Spencer, R.G.S., and Simonneaux, G. (1999) Proton nuclear magnetic resonance study of the binary complex of cytochrome P450cam and putidaredoxin: Interaction and electron transfer rate analysis. *FEBS Letters* 455, 302-306.

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Schlichting, I., Jung, C., and Schulze, H. (1997) Crystal structure of cytochrome P-450cam complexed with the (1S)-camphor enantiomer. *FEBS Letters* 415, 253-257.

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Modeling Nucleic Acid Structure and Recognition by Proteins

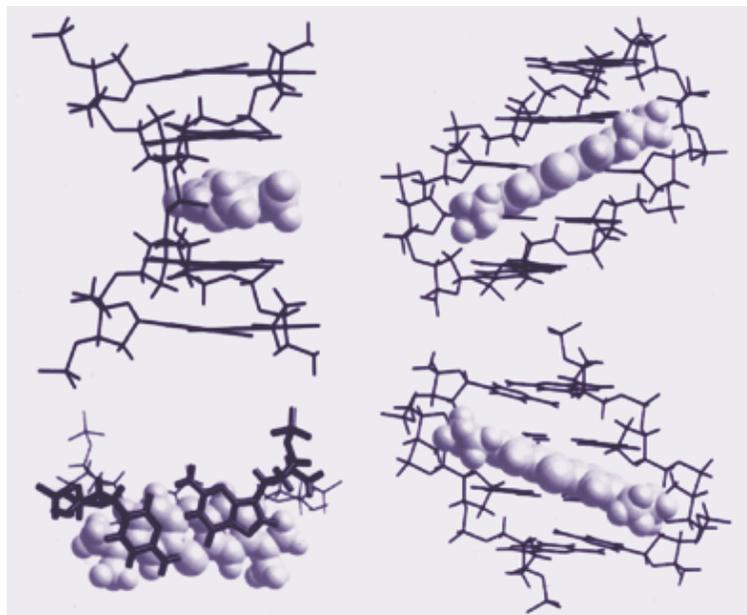
Heinz Sklenar

Understanding nucleic acid structures is important due to the variety of biological functions fulfilled by DNA and different classes of RNA molecules. Investigations using computer simulations are based on physical models that have been developed in order to describe the driving forces for the formation of molecular structures. Such studies are very different from laboratory experiments, yet the capability for exploring the large diversity of possible structures, and of their stability under given conditions, are often complementary. 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. New algorithms using a continuum treatment of solvent electrostatic effects in combination with molecular force field models have enabled us to apply conformational search techniques for structural predictions. 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.

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 the selective recognition of specific base sequences by regulatory proteins. The structural libraries, based on our modeling results and the analysis of experimental structures, permit fast conversion of base sequences into profiles of structural parameters. Thus, both systematic structural analysis of binding sites for specific transcription factors, and the search for sites with characteristic and common features in long sequences with unknown function, have become possible. The applicability of this approach to the characterization of individual regulatory elements has been confirmed. Recent data suggest that there are specific examples of transcription factor – DNA interactions where consideration of structural features gives significant insight into our understanding of the recognition of regulatory elements compared with pure statistical sequence analysis.

Figure 25: Three binding modes of the photoactive methylene blue molecule with DNA: intercalation (left, views perpendicular and parallel to the helical axis), minor groove binding (right, upper panel), and major groove binding (right, lower panel).



Conformation and stability of non-canonical structural motifs in DNA and RNA

The remarkable intrinsic stability of certain classes of structural motifs and their re-occurrence in many RNA structures indicate that they play an important role in tertiary folding and in biological functions of RNA molecules. The emerging “tool kit” of RNA structural motifs will help us understand better the relationships between sequences, structures, and functions, and is also expected to substantially aid model building of RNA 3D-structures. A computational approach to this task would be helpful, provided that any experimental data set can be reliably extended. A force-field based conformational analysis has been applied to single-base bulges, GNRA tetraloops, and the asymmetric internal E-loop. The structures are represented by an ensemble of conformers that were selected purely on the basis of calculated free energies from a large set of conformations generated by a systematic combinatorial loop search. In contrast to the well-known failure of such predictions based on quasi-vacuum force fields, the inclusion of reaction field contributions by the solvent results in a selection of low-energy conformers in accordance with experimental data. Besides the detailed atomic resolution structure an understanding of the mobility and conformational deformability of RNA structures is important for interpreting its function. Currently, only some

aspects of the dynamic behavior of nucleic acids can be measured experimentally. The harmonic-mode analysis method has been used to characterize the conformational deformability of regular Watson-Crick paired, mismatch and bulge containing RNA fragments.

DNA – ligand interactions

Photoactive methylene blue is one of the most efficient singlet-oxygen generating dyes. It binds to DNA and induces photosensitized reactions which can be used for sequence-specific cleavage of the DNA backbone. Photophysical data obtained for methylene blue in complexes with DNA indicate different binding modes of the dye depending on the base sequences. Based on a modeling study of methylene blue binding to a DNA decamer with an alternating GC base sequence, we have derived six structural models. The conclusion of the detailed structural and energetic analysis is that these structures can be considered as representatives of methylene blue – DNA complexes in different binding modes (intercalation, minor and major groove binding). The predicted structures may be useful for a more detailed interpretation of experimental results and could serve as a starting point for studying base sequence effects in view of the photochemical applications of methylene blue in gene therapy.

Classification of C2H2 zinc finger proteins in the *C. elegans* genome

C2H2 zinc fingers, short repetitive sequence modules in zinc finger proteins, are the most frequent nucleic acid binding motif in eukaryotic genomes. Their high sequence variability, combined with different arrangements of the fingers, results in the rather diverse functions of the zinc finger proteins, ranging from sequence-specific binding to DNA or DNA/RNA hybrids, binding to RNA or heteroduplex DNA, to their involvement in protein – protein interactions. Using a substantially improved sequence search pattern, the complete set of C2H2 zinc finger proteins has been identified in the *C. elegans* genome. We have detected zinc fingers in about 1 % of all ORFs. Our attempts at a functional classification are based on known data of zinc finger – DNA recognition, on the discovery of several *C. elegans* zinc finger proteins homologous to functionally characterized zinc finger proteins in other species and on sequence pattern analysis using our zinc finger protein databases. In earlier studies, a similar approach was applied to the complete set of yeast zinc finger proteins.

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Conformation, Stability and Interaction of Biological Macromolecules

Heinz Welfle

Knowledge of the conformation, stability and interactions of biological macromolecules is a basic prerequisite for understanding the fundamental problems of molecular biology. We are studying the physicochemical properties of selected targets, such as enzymes, transcriptional and translational factors and their complexes with nucleic acids, and antibodies and antibody-peptide complexes. Our main tools are circular dichroism, fluorescence, infrared and Raman spectroscopy and calorimetric methods.

Interaction of tetracyclin-repressor with operator DNA

in collaboration with Wolfram Saenger, Free University of Berlin

Tetracyclin repressor (TetR) is involved in the most common mechanism of tetracycline resistance of Gram negative bacteria. We have analysed the interaction of the class D TetR protein with an oligodeoxyribonucleotide with a sequence corresponding to operator site O1. Infrared and Raman spectroscopy were employed to investigate the solution structure of TetR, and the TetR:operator complex was studied by Raman spectroscopy. The following results were obtained for H₂O and D₂O samples: i) The B-DNA conformation of the operator site is conserved in the complex with only small perturbations of the backbone geometry; ii) TetR and operator DNA interact at major-groove sites; iii) Minor changes in TetR secondary structure occur on operator binding; iv) Local environments of aromatic amino acids are altered. These data are consistent with a model based on genetic and biochemical experiments.

Antibody-peptide interaction

in collaboration with Wolfgang Höhne and Jens Schneider-Mergener, Humboldt University, Berlin

High affinity antibodies are usually thought to be monospecific, nevertheless, polyspecificity is frequently observed. 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. Using a synthetic positional scanning combinatorial library, five unrelated peptides have been identified that compete with each other for binding to the paratope region of the antibody (A. Kramer, T. Keitel, K. Winkler, W. Stöcklein, W. Höhne, J. Schneider-Mergener (1997), Cell 91, 799-809).

The crystal structures of the CB4-1 Fab fragment alone and in complex with epitope-homologous and non-homologous peptides have been solved at 2.6 Å resolution (T. Keitel, A. Kramer, H. Wessner, C. Scholz, J. Schneider-Mergener, W. Höhne (1997) Cell 91, 811-820). CD spectra of the antibody and its Fab and Fc fragments exhibit the characteristics expected of β -proteins. Lowering the pH to 3.5 reduces the stability but does not change the conformation whereas, between pH 3.5 and 2.0, conformational changes occurred and new, acid-induced and surprisingly thermostable forms are present. To understand the main determinants of the complex formation between CB4-1 and peptides, we are studying the binding reaction by isothermal titration calorimetry. This method provides a complete thermodynamic description of the reaction. Binding constants, binding stoichiometry and binding enthalpies have been obtained experimentally and allow calculation of binding free energies and binding entropies. For the CB4-1-peptide interaction, the enthalpy and entropy contributions to the free energy differ significantly from peptide to peptide but in each system studied so far the complex formation is enthalpically driven.

Translational initiation factor IF2 from *Bacillus stearothermophilus*

in collaboration with Claudio O. Gualerzi, University of Camerino, Italy, and Udo Heinemann, MDC

Initiation factor IF2 is involved in the initiation step of eubacterial translation, and its main recognised function is the correct positioning of initiator fMet-tRNA^{fMet} in the ribosomal P site. To accomplish its function in translation, IF2 interacts with fMet-tRNA^{fMet} via its C-terminal domain (IF2 C) and with GTP/GDP and 50S ribosomal subunits via its central G-domain. Our efforts have concentrated on elucidating the structure of IF2 C and the molecular nature of its interaction with fMet-tRNA^{fMet}. Recently, we have shown in thermal and guanidinium chloride-induced unfolding studies that IF2 C consists of two subdomains. Isolated subdomain IF2 C-2 binds fMet-tRNA^{fMet} with the same specificity and affinity as native IF2. IF2 C-2 has been identified as a globular molecule containing predominantly structures (25% antiparallel and 8% parallel strands) and turns (19%) whose structural properties are not markedly affected by the presence or absence of the N-terminal subdomain IF2 C-1. Functional and structural characterisation of Cys mutants of IF2 C have provided evidence that I) both Cys residues are buried within an hydrophobic core; II) neither Cys is functionally essential; III) both Cys residues are located near the active site, probably without participating directly in fMet-tRNA binding.

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- Förster, C., Krafft, C., Welfle, H., Gualerzi, C.O., and Heinemann U. (1999) Preliminary characterization by X-ray diffraction and Raman spectroscopy of a crystalline complex of *Bacillus stearothermophilus* initiation factor 2 C-domain and fMet-tRNA^{fMet}. *Acta Cryst. D55*, 712-716.
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Protein Structure Analysis and Proteome Research

Brigitte Wittmann-Liebold

The research of the group has concentrated on the structure and function of proteins and protein complexes and their protein-protein and protein-RNA interactions at the molecular level. In addition to own projects, several long-term collaborations with medical groups of the MDC and the Robert Rössle Clinic (the groups of Dr. Kurt Bommert and Dr. Peter Daniel / Department Prof. B. Dörken) have been started in order to study apoptosis-induced processes and the protein complexes involved in transport, signalling and developmental processes (in collaboration with Dr. P.M. Kloetzel, HU Berlin and R.A. KroczeK, Robert Koch Institute, Berlin). Proteome research has been successfully carried out on several projects, and proteins associated to myocardial disease and apoptosis-associated processes have been identified. These studies have

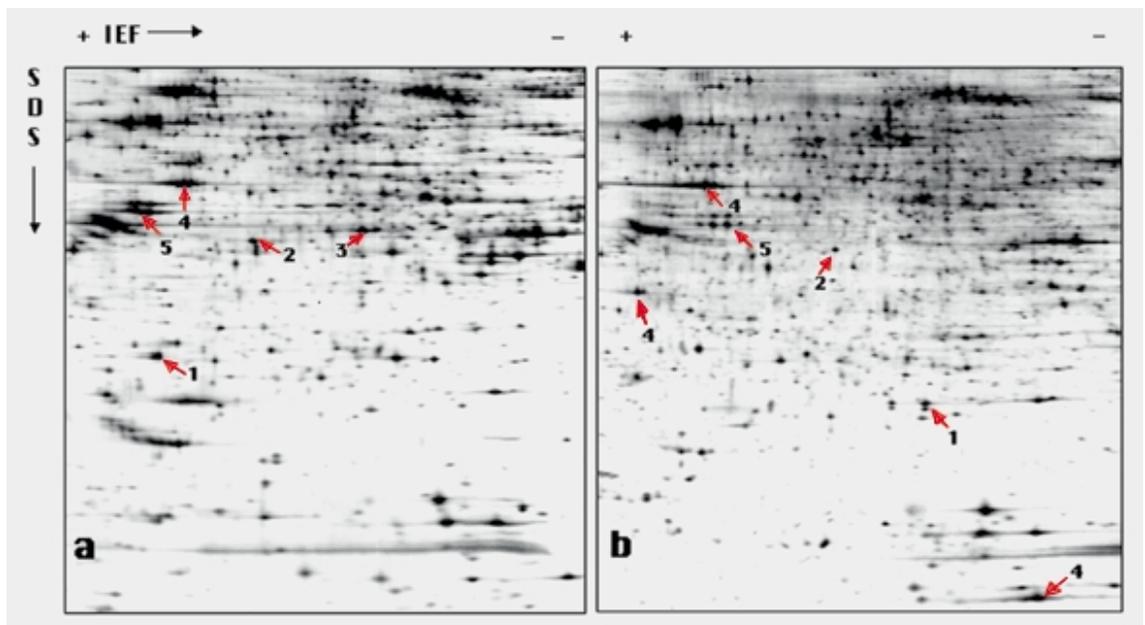
yielded valuable data on the total protein expression in the cell in disease and under developmental conditions. Our crosslinking data from ribosomes have established direct contact sites between the RNA and binding proteins which have allowed insertion of the 3D-structures of these proteins into recent eubacterial RNA fine structure models (Brimacombe et al., in press).

The large increase in molecular data obtained by proteome analysis has been achieved by the application of highly sensitive protein 2D-electrophoresis isolation techniques in combination with MALDI-mass fingerprinting and nanospray-ESI-TOF spectrometry. Mass fingerprinting provides information on the masses of the peptides derived from the individual proteins allowing us to perform searches in the databases for protein identification. This yields 40-80% sequence coverage for most of the protein spots. In addition, using nanospray-ESI-TOF mass spectrometry, 4-8 partial peptide sequences can be derived from the peptide mixture with minute sample amounts (< 1pmol), and these data make protein identification even more reliable. After separation of the complex total cell protein mixture by high-resolution 2D-electrophoresis in 24x32 cm gels up to 5000 proteins can be resolved (Klose and Kobalz, 1995). Then, the proteins of interest are excised from the gel, cleaved *in situ* by trypsin, desalted and the peptide mixture is subjected to mass

spectrometry. Often the identification of the parent protein is possible from these masses, or alternatively, partial sequence information helps us assign the correct protein. In this way, it is possible to identify many proteins overexpressed or reduced after IgM apoptosis induction of Burkitt lymphoma BL60 cells (Müller et al., 1999). Among these, several new proteins have been detected which so far are not connected with any of the apoptosis processes. Their genes are now being cloned using appropriate partial peptide sequences and completely sequenced. This will also permit recombinant protein isolation for further functional assays.

In addition, using highly sensitive protein analytical methods in combination with truncation experiments, sequences within the proteasomal prosequences have been deduced which mediate efficient integration of β -subunits into the 20S-proteasome complex (Schmidt et al., 1999). Employing human and yeast proteasomes, the function of the proteasome regulatory particle has been studied. These exhibit chaperone-like activities as revealed by native citrate synthase recovery (Braun et al., 1999). Moreover,

Figure 26: Silver stained 2-DE analytical gels of the proteins derived from Burkitt lymphoma BL 60 cells: a) normal cells, b) cells after anti-IgM-induced apoptosis. Examples of proteins altered after treatment: 1-D4-GDI, fragmented in b), 2-ribosomal protein Po, pl shift in b), 3-neutral calponin (not found in the apoptotic gel), 4-actin and actin fragments, 5-hnRNP C1/C2, pl shift in b).



besides the known T-cell specific surface receptors CD28 and CTLA-4, a third member of this family, the inducible co-stimulator (ICOS) has been identified (Hutloff et al., 1999) and results indicate that ICOS is another major regulator of the adaptive immune system.

In recent years it became obvious that genome analysis alone cannot establish structural-functional correlations between biomolecules in various cell processes. On the other hand, the complete description and analysis of all proteins within a cell, cell line or microorganism (proteome analysis) allows us to study dynamic states within the cells, e.g. to get clues about cell development, proliferation, and regulation. We have been able to demonstrate the potential of the highly sensitive protein analysis tools available now. These allow advanced studies in the analysis of signal transduction events, tumor development, drug screening, and protein marker assignment for early diagnosis. Great efforts have been made to make the group one of the world leaders in proteome research.

Selected Publications

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Prof. Dr. Theodora Choli-Papadopoulou, Thessaloniki, Greece
Prof. Dr. Tsezi Egorov, Moscow and coworkers
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Cell Growth and Differentiation

Cell Growth and Differentiation

The elucidation of mechanisms of cell growth and differentiation provides the basis for understanding aberrant processes that occur in cancer and cardiovascular diseases. The genetic information contained in all cell types of the human body, such as hepatocytes, blood cells and cardiomyocytes, is basically identical. Differences in the appearance and function of these cells are then generated during embryonic development through a range of differentiation processes. Moreover, cell growth and cell death (apoptosis) are two basic principles of development and homeostasis that are under stringent regulation. In pathophysiological situations, like cancer and cardiovascular diseases, these basic mechanisms become deregulated. Cancer cells grow in an uncontrolled fashion. They fail to terminally differentiate, and they penetrate surrounding tissue and migrate to distant sites in the body where they do not belong, i.e., they become invasive and metastatic. Disturbances of growth and differentiation also play crucial roles in cardiovascular diseases: de-differentiation and proliferation of smooth muscle cells can cause a severe reduction in the vessel lumen. Moreover, the inability of cardiomyocytes to proliferate prevents tissue regeneration after injury to the heart.

The sequence of the entire human genome will soon be available and a future goal will be to understand the implications of these gene sequences with respect to normal developmental processes and to the development of disease. It will become possible to understand not only the function of single genes, but also to identify entire cascades of genes whose products interact and regulate each other. Research directed at obtaining a comprehensive and overall view of these subjects is termed Functional Genomics. Functional Genomics, which is the key interest of our department, complements the in-depth, molecule-by-molecule, pathway-by-pathway studies carried out by many molecular and cell biology laboratories around the world.

Today, we know that cancer is a disease of a distinct set of malfunctioning genes. Malfunction occurs by mutations in these genes by two types of mechanisms: activation of genes that support cell growth (proto-oncogenes), and inactivation of genes that arrest cell growth (tumor suppressor genes). Genes involved in the control of growth and differentiation and in cancer have been found to function in I) signal transduction (exemplified by the ras oncogene, which is part of the signaling cascade of tyrosine kinase receptors and plays a role in 20 - 30 % of human tumors), II) in gene regulation (like the myc oncogene), III) in maintenance of genomic stability and control of apoptosis (like the p53 tumor suppressor gene, which is mutated in approximately 50 % of all human tumors), or IV) in preservation of normal tissue integrity (like the APC tumor suppressor gene, which is mutated in 80 % of colon carcinomas).

In the Cell Growth and Differentiation Program of the MDC, several groups are studying mechanisms of action of proto-oncogenes and tumor suppressor genes that function in the control of signal transduction and gene regulation. Among the studies performed at the MDC, it has been found that the transcription factor C/EBP β specifically interacts with the SWI/SNF chromatin remodelling complex, and that this interaction is required to activate a group of myeloid genes in collaboration with the Myb proto-oncogen product. Grafting the N-terminus of C/EBP β onto Myb generates a chimeric transcription factor that recruits SWI/SNF and activates chromosomal genes, even in the absence of C/EBP. This shows that SWI/SNF recruitment is an important feature of the Myb-C/EBP collaboration, and it is the first demonstration that in vertebrates the SWI/SNF complex may be recruited by transcription factors to remodel chromatin at distinct sets of genes (Kowenz-Leutz, E. and Leutz, A. Molecular Cell 4, 735-743, 1999). A potential new tumor suppressor gene, conductin, has been identified, and it has been shown that conductin induces the degradation of β -catenin and blocks wnt signaling. Conductin is a scaffold protein which assembles a multiprotein complex by binding to β -catenin, the tumor suppressor gene product APC and the serine/threonine kinase GSK3 at separate domains. Complex formation is responsible for the phosphorylation of β -catenin by GSK3 β which leads to ubiquitination of β -catenin and its subsequent destruction by the proteasome (Behrens et al. Science 280, 596-599, 1998). NF- κ B is an important survival factor of tumor cells. It has been found that I κ B kinases phosphorylate NF- κ B p105 in the cytoplasm and that this results in rapid degradation of p105 by the proteasome. NF- κ B p50 which is formed by processing of p105, is liberated and transported to the nucleus. Since p50 homodimers are specifically bound by the oncoprotein Bcl-3, TNF α or IL-1 stimulation results in rapid accumulation of transcriptionally active p50-Bcl-3 complexes in the nucleus (Heissmeyer et al. EMBO J. 18, 4766-4778, 1999). To test the function of the chemokine receptor CCR7, mice were generated in which the CCR7 locus has been disrupted by gene targeting. Lymph nodes (LN) of CCR-7 deficient mice were found to be devoid of naive T cells and

dendritic cells (DC). Adoptive transfer experiments to wild-type recipients has demonstrated that the migration of CCR7-deficient T cells and B cells into LN, Peyer's patches, and spleen is severely hampered. The overall disturbed microarchitecture of secondary lymphoid organs, caused by the impaired entry and retention of lymphocytes and antigen-presenting DC, may explain why CCR7-deficient mice fail to mount a rapid primary B or T cell response (Förster et al., Cell 99, 23-33, 1999).

Disturbances of growth and differentiation also play a crucial role in cardiovascular diseases. Cardiac myocytes build the contractile apparatus of the heart and respond to increased work load by an increase in cell numbers (proliferation) during fetal development. However, soon after birth cardiomyocytes lose their capacity to proliferate and only respond to changing physiological needs by an increase in cell size (hypertrophy). Although adaptive by nature, this hypertrophic response can ultimately lead to heart failure. The permanent withdrawal from the cell cycle (terminal differentiation) efficiently protects cardiac myocytes against malignant transformation, but also prevents tissue regeneration after injury to the heart. In contrast, vascular smooth muscle cells (VSMC) maintain the ability to change between a proliferative and a differentiated, non-proliferative, state throughout their life. This allows efficient damage repair after injury as well as the formation of new or larger vessels, but can also become a major clinical problem as it contributes to the reduction in the vessel lumen (stenosis). Interventional procedures (angioplasty) to restore normal vessel lumen frequently fail as they elicit a proliferative response of the surrounding VSMC leading to repeated lumen loss (restenosis).

The ability of vascular smooth muscle cells, as well as of endothelial cells to proliferate and form new vessels is also crucial for tumor development, since the supply of nutrients and oxygen is required to sustain the uncontrolled growth of cancer cells. Several groups at the MDC are studying the differentiation and cell cycle regulation of smooth muscle cells and cardiac myocytes to develop specific approaches to control the growth and differentiation of these cells in cardiovascular disease. Other groups are investigating the regulation of the contractile apparatus of muscle cells and the role of the calcium homeostasis in healthy and diseased hearts. Transgenic and experimental animal models have been established as disease models to develop novel strategies to combat cardiovascular dysfunctions.

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Growth Control and Gene Regulation in the Hematopoietic System

Achim Leutz

Red and white blood cells originate from hematopoietic stem cells located in the bone marrow. Stem cells give rise to progenitors that may differentiate into one of at least eight hematopoietic cell types, such as erythrocytes, neutrophils, or macrophages. Hematopoietic proliferation, differentiation, and leukemogenesis are intimately linked to a number of key transcription factors that regulate expression of lineage-specific genes or entire developmental programs. Dysregulation of the pathways that control the expression or activity of critical transcription factors, e.g., by mutation or viral interference, may cause various diseases such as immune defects, anemia, or leukemia. Thus, hematopoiesis provides striking opportunities to address both fundamental biological questions and clinically relevant issues such as: How are cell growth and differentiation regulated? How are proliferation and differentiation connected? How is cell identity achieved during lineage commitment? Obtaining answers to these questions will improve our understanding and treatment of many diseases. We have set out to determine how gene regulatory proteins control cellular growth and differentiation programs and how their dysregulation may cause disease.

A bipartite gene switch

Proteins of the CCAAT/Enhancer Binding Protein family (C/EBP) induce expression of genes which account for myelomonocytic commitment, differentiation, and proliferation arrest. This became evident when a conditional nuclear receptor-C/EBP chimera was expressed and activated in progenitor cells that subsequently induced their differentiation into eosinophils. In collaboration with the cellular Myb proto-oncoprotein (c-Myb), C/EBPs even activate myeloid genes in heterologous cell types, e.g., in fibroblasts. Such combinatorial gene switches permit plasticity during growth and differentiation and limit the number of regulators and pathways required for cell type specification. The concept of concerted action of transcription factors has now been confirmed by many research groups and has been extended to other hematopoietic transcription factor interactions.

Chromatin remodeling and lineage-specific gene expression

A prerequisite for ectopic activation of silent genes, such as myeloid genes induced by Myb plus C/EBP in fibroblasts, is to overcome the repressive effects of chromatin. This is accomplished by large protein complexes that locally remodel chromatin. An assay that we have established to monitor activation of endogenous, chromatin embedded genes has helped to unravel the mechanism of the collaboration between Myb and C/EBP β . It became evident that C/EBP β specifically interacts with the SWI/SNF complex, and that this interaction is required to activate a group of myeloid genes. An amino-terminal peptide which is contained only in one particular isoform of C/EBP β (see below), is required for SWI/SNF recruitment. Grafting the N-terminus of C/EBP β onto Myb generates a chimeric transcription factor that recruits SWI/SNF and activates chromosomal genes, even in the absence of C/EBP β . This shows that SWI/SNF recruitment is an important feature of the Myb- C/EBP β collaboration. It is also the first demonstration in vertebrates that the SWI/SNF complex may be recruited by transcription factors to remodel chromatin at distinct sets of genes.

Cell growth arrest and differentiation

In addition to inducing differentiation, C/EBPs arrest cells in the G1 phase of the cell cycle. To understand how C/EBPs mediate both proliferation arrest and differentiation, we investigated whether oncoproteins could interfere with distinct C/EBP functions. Of various oncogenes examined, only E7, from the high-risk papillomavirus type 16 or 18 strains, abrogated C/EBP α -induced growth arrest. Remarkably, E7 did not interfere with differentiation, suggesting that the two C/EBP functions can be separated (see figure). Since C/EBPs are expressed in mammary epithelium, cervical epithelium and skin, our results imply that elimination of C/EBP-mediated proliferation arrest might contribute to papilloma pathology. Furthermore, the results suggest that C/EBPs act as tumor suppressor proteins and, therefore, are targets of tumorigenesis.

GBX2 is a homeobox target gene of Myb

The product of c-Myb regulates genes involved in stem cell self-renewal and in progenitor differentiation. It is, therefore, important to identify critical Myb target genes and determine their function. Recently, we isolated the homeobox gene GBX2 as a target of Myb. Ectopic expression of GBX2 in precursor cells changes their phenotype and growth properties suggesting that GBX2 is involved in hematopoiesis and the establishment of the transformed phenotype by the Myb onogene.

GBX2 gene expression is directly induced by a leukemogenic version of Myb, whereas its activation by c-Myb depends on a co-activated receptor tyrosine kinase or ras pathway. Thus, leukemogenic Myb represents a gain-of-function derivative of its cellular counterpart. Moreover, the results suggest that a signaling cascade regulates c-Myb function.

Activation of GBX2 by c-Myb depends on signaling from the cell surface. This is of particular interest since the *Drosophila melanogaster* homologue of GBX2, the *unplugged* gene, is downstream of the FGF receptor during tracheal development. This implies that regulation of GBX2 expression is part of a conserved

developmental pathway that may involve the Myb onco-protein. In support of such a speculation are our observations that murine GBX2 and FGF-2 knock-outs display epistatic hematopoietic defects and GBX2 and FGF-2 are co-expressed in hematopoietic cell lines. We are, therefore, searching for a link between GBX2, FGF-2, its receptor, and Myb.

Interestingly, the same mutations in leukemogenic Myb that constitutively activate GBX2 concomitantly abrogate the collaboration between Myb and C/EBP. Accordingly, they are loss-of-function mutations for C/EBP collaboration. Since C/EBP induces cell differentiation and proliferation arrest, it appears that the oncoprotein abolishes the function of a genetic switch that controls terminal differentiation of myeloid cells.

Translational regulation of transcription factors

Several protein isoforms arise from both GBX2 and C/EBP mRNAs by alternative initiation of protein translation at different start codons. The isoforms give rise to DNA regulatory proteins with entirely different functions. In the case of C/EBPs, full-length proteins are trans-activators while an internally initiated protein is a repressor. The C/EBP transactivator proteins mediate proliferation arrest and cellular differentiation, whereas the repressor permits proliferation. Long and short protein isoforms are also generated from the GBX2 mRNA. Unlike C/EBPs, however, long GBX2 isoforms are repressors whereas the short form is an activator. The activator GBX2 supports expression of at least one cytokine that promotes precursor cell proliferation. Thus, internal start site usage will support growth because short, growth-promoting isoforms replace the long,

differentiation-promoting isoforms of C/EBP and GBX2. In contrast, preferential initiation from the first start codons will support differentiation by increasing the pool of long isoforms. Site-directed mutagenesis has revealed that translation initiation control relies on a highly conserved small upstream open reading frame (uORF). We have now begun a detailed analysis of the relationship between GBX2 and C/EBP isoform expression, translation initiation factor activity, regulation by uORF, and the biological functions of protein isoforms. From our results, we found that two differentially initiated C/EBP β isoforms display striking differences in recruitment of the SWI/SNF complex. It is anticipated that pathways and factors involved in the control of translational initiation are important regulators of hematopoiesis and may be novel targets for innovative drug therapies.

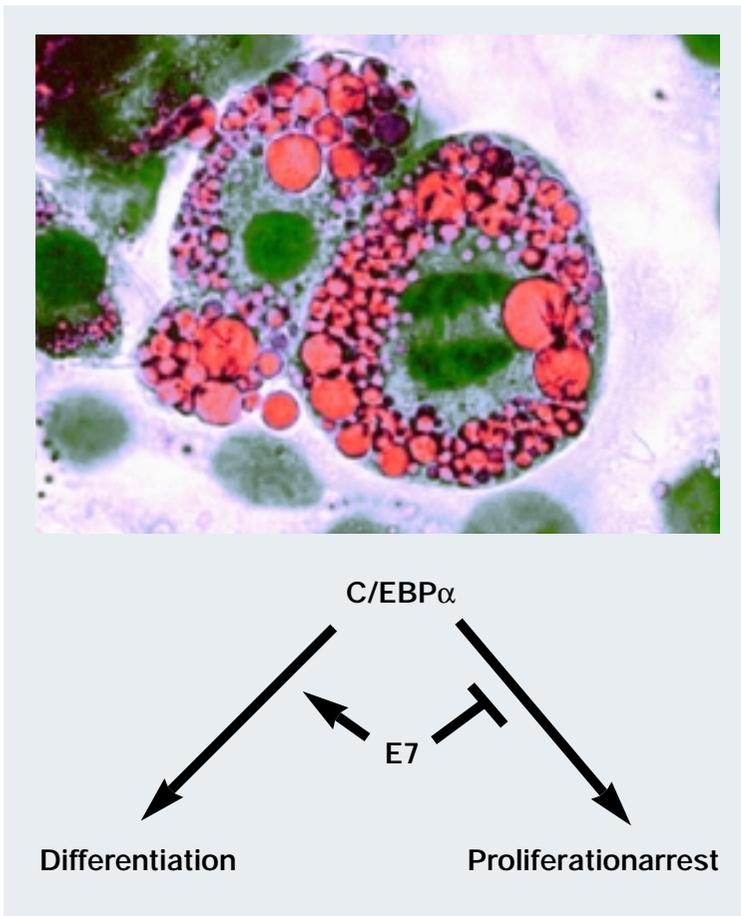


Figure 27: Proliferation in terminally differentiated adipocytes is induced by the E7 oncogene. Cell division is evident by separating metaphase chromosomes and terminal fat cell differentiation by storage of fat droplets in the cytoplasm. The model underneath indicates that the E7 oncoprotein uncouples C/EBP programs for proliferationarrest and for differentiation.

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Patent Application

198 59 780.0

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Regulation of Transcription in Mammalian Cells

Claus Scheidereit

Cellular growth and differentiation largely depends on the expression of sets of genes which are controlled essentially at the level of transcription. Differential gene expression patterns are programmed by transcription factors, whose activity in turn is modulated by complex networks of signal transduction cascades. The main objective of our laboratory is to understand how signal transduction processes are coupled to transcription. A model system with wide physiological and medical relevance is nuclear factor kappaB (NF- κ B) and its co-regulators and accessory proteins. A major goal of our research is to understand the structures and mechanisms underlying gene regulation by this complex system.

Pathways and structures that regulate NF- κ B activity

The pleiotropic transcription regulator nuclear factor κ B (NF- κ B) plays an important 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 cellular growth or programmed cell death. In its inactive, latent form NF- κ B is kept in the cytoplasm by association with I κ B molecules, which inhibit nuclear translocation and DNA binding of NF- κ B. Stimulation of cells with a variety of agents, such as bacterial lipopolysaccharides (LPS), phorbol esters (PMA), tumor necrosis factor α (TNF α), interleukin-1 (IL-1) or UV light results in the proteolysis of the I κ B molecules and liberation of active NF- κ B into the nucleus. Induced I κ B proteolysis is triggered by I κ B phosphorylation mediated by an I κ B kinase (IKK) complex, which is activated by the many NF- κ B-stimulating pathways. The composition and regulation of the IKK complex is under investigation and, of particular interest, is the identification of molecules which directly activate or inhibit the complex.

Differential regulation of NF- κ B activity by I κ B α , I κ B β , p105 and Bcl-3

The mammalian NF- κ B family consists of five members, p50, p65, p52, the protooncogene product c-Rel and RelB. These conserved proteins form various hetero- and homodimers and are bound by I κ B molecules I κ B α , β , and ϵ , the I κ B-like precursor proteins for p50 and p52, p105 and p100, respectively, or by the nuclear I κ B homologue Bcl-3. We have found that in human cells I κ B β is expressed as two distinct splicing variants, I κ B β 1 and I κ B β 2. While both forms equally well associate with NF- κ B, they differ in their responsiveness to signals and sub-cellular localization. Due to a lack of components of a carboxyterminal PEST sequence, I κ B β 2 is only weakly degraded in response to inducing agents and so its relative abundance determines the responsiveness of a given cell. In B lymphocytes, I κ B β 1, but not I κ B β 2, is found in the nucleus and may contribute to the persistent NF- κ B

activity in these cells. These findings may also indicate that the efficiency of the IKK complex, which phosphorylates I κ B β 1 and 2 at invariant aminoterminal residues, is affected by the presence of the PEST domain. The IKK complex phosphorylates I κ B β and I κ B α at a conserved signal response domain and this sequence, containing also lysines for phosphorylation-dependent ubiquitin-conjugation, is sufficient to confer inducible degradation. A short 50 amino acid sequence of I κ B α , when fused to other proteins, triggers degradation of these proteins when cells are activated by TNF α or other agents which activate IKKs. We have also found that the NF- κ B precursor proteins, p105 and p100, which on processing give rise to p50 and p52, sequester other NF- κ B subunits including their processing products in the cytoplasm and so act like I κ B molecules. On stimulation with NF- κ B activating agents, cellular p105 is phosphorylated by same kinetics as I κ B α . We have now found that IKKs phosphorylate p105 and that the major sites are three serines close to the carboxyterminal end of p105. Phosphorylation at these sites by IKKs results in rapid, complete degradation of p105 by the proteasome. p105-associated NF- κ B subunits, such as p50, which is formed by processing of p105, are liberated and are transported to the nucleus. Thus, in parallel with the release of NF- κ B dimers by induced degradation of I κ B α or I κ B β 1, other NF- κ B subunits, including p50 homodimers, are released by p105 degradation. Since p50 homodimers are specifically bound by the nuclear I κ B homologue Bcl-3, TNF α or IL-1 stimulation results in rapid accumulation of p50-Bcl-3 complexes in the nucleus. The protooncogene product Bcl-3 acts like a transcriptional co-activator for p50 homodimers, which lack their own transactivation domains. Several nuclear cofactors and chromatin-modifying proteins have been identified which potentiate the transcription activation potential of Bcl-3-p50 complexes. These accessory proteins include the histone acetylase, Tip60, which superstimulates Bcl-3-mediated transcriptional activation and forms quarternary complexes with p50-Bcl-3 bound to promoter DNA.

Requirement of NF- κ B for growth and survival of lymphoma and leukemia cells

In collaboration with the research group of B. Dörken, we have discovered the crucial role of constitutive nuclear NF- κ B activity in the viability of malignant cells in Hodgkin's disease (HD). NF- κ B counteracts programmed cell death and, hence, may critically contribute in the etiology of HD. Similarly, antiapoptotic effects of NF- κ B have been demonstrated by other groups in transformed cell lines, primary murine cells or breast cancer cells.

Constitutive NF- κ B activity is further required for cell cycle progression of HD cells. However, proliferation of virally transformed cell lines with an inactivated retinoblastoma protein (pRB) checkpoint do not require NF- κ B activity. In collaboration with the group of M. Strauss, we can now demonstrate with primary non-transformed cells that NF- κ B is, in fact, also required for growth factor signaling in normal primary cells and promotes G₁ to S phase transition by regulating the RB pathway. NF- κ B activates transcription of the cyclin D1 promoter in response to serum stimulation and, thereby, contributes to pRB phosphorylation. Further functional connections between NF- κ B and cell cycle regulator proteins are under investigation.

A characteristic feature of HD cells is the constitutive presence of NF- κ B p50-p65 in the nucleus. Our recent analysis of Hodgkin cells has shown that the NF- κ B/I κ B system is dysregulated in a cell-autonomous manner, involving both mutations of I κ B genes and aberrant activation of the IKK complex. Similar constitutive NF- κ B activation has been found in acute lymphoblastic leukemia (C-ALL), again caused by IKK activation. Further studies are being performed to elucidate the mechanism of constitutive NF- κ B activation.

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Differentiation and Growth Control in Lymphocyte Development and Function

Martin Lipp

The identification and functional analysis of differentiation and growth control genes in lymphocyte development will improve our understanding of 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 projects:

- I) role of chemokines and chemokine receptors in lymphocyte migration, organogenesis of lymphoid tissues and 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.

Functional organization of lymphoid organs by the chemokine system

Chemokines are small basic proteins which exert their chemoattractive activities via binding to seven-transmembrane-domain receptors signaling through heterotrimeric G proteins. Chemokines and their receptors can be broadly divided into two functionally distinct categories. On one hand, inflammatory chemokines, induced or upregulated by inflammatory stimuli, are responsible for recruiting cells involved in acute inflammatory reactions; on the other, constitutive chemokines, produced in bone marrow, thymus and secondary lymphoid organs, are responsible for the homeostatic control of leukocyte traffic and for mediating encounters between cells that need to interact to generate an immune response. Our recent finding, that the chemokine receptor BLR1/CXCR5 is needed for B cell migration into lymphoid follicles, is the first experimental evidence that the chemokine system plays an essential role as a regulator of migration of lymphocyte subsets and is involved in the functional compartmentalization of lymphoid organs.

Generation of monoclonal antibodies specific for human CCR7 revealed expression of CCR7 on peripheral T cell subsets, B lymphocytes and monocytes. Furthermore, whereas CCR7 was not detected on monocyte-derived immature dendritic cells (DC), surface expression of CCR7 was gradually up-regulated following *in vitro* induced maturation of DC. To test the function of CCR7, we produced mice whose CCR7 locus had been disrupted by gene targeting. Lymph nodes (LN) of CCR7-deficient mice are devoid of naive T cells and DC and adoptive transfer experiments to wild-type recipients demonstrated that the migration of CCR7-deficient T cells and B cells into LN, Peyers patches, and spleen was severely hampered. Therefore, the overall disturbed microarchitecture of secondary lymphoid organs, caused by the impaired entry and retention of lymphocytes and antigen-presenting DC, may explain why CCR7-deficient mice fail to exhibit a rapid primary B or T cell response. In collaboration with A. Lanzavecchia, Basel, we have shown that the memory response is mediated by two distinct T cell

subsets: tissue-seeking CCR7⁻ effector memory T cells (T_{EM}) provide immediate protection in inflamed tissue while lymph node-seeking CCR7⁺ central memory T cells (T_{CM}) provide help for DC and B cells and generate a new wave of effector cells. Thus, by bringing together lymphocytes and DC to form the characteristic microarchitecture and functional microenvironments of secondary lymphoid organs, the homeostatic chemokine system has been shown to be an important regulator of lymphocyte homing and, consequently, functions as a coordinator for initiating an antigen-specific immune response and creating immunological memory.

Immune modulatory and growth-inducing functions of viral chemokine receptors

We have previously shown that Epstein-Barr-Virus (EBV) specifically transactivates expression of the cellular chemokine receptor CCR7 by its regulatory nuclear factor EBNA2. In contrast to EBV, several other human herpesviruses, like cytomegalovirus or the lymphotropic human herpesviruses type 6 (HHV-6) and Kaposi's sarcoma-associated herpesvirus (KSHV), also termed HHV-8, encode viral chemokine receptors and chemokines in their genome suggesting that herpesviruses use the chemokine system to interfere with the growth and differentiation program of the host and subvert specific immune responses. Epidemiological and molecular evidence has linked infection with KSHV 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 is aimed at establishing whether the KSHV-encoded chemokine receptor (KSCR), which is known to be constitutively activated and able to induce proliferation, plays a role in the development of human herpesvirus 8-associated diseases and malignancies as an essential oncogenic or paracrine factor, or both. 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.

Role of lysosphingophospholipid receptors in the immune system

We have cloned human EDG6 from *in vitro* differentiated dendritic cells in order to identify novel G protein-coupled receptors (GPCR) that control immune functions. EDG6-specific RNA was detected almost exclusively in lymphoid and hematopoietic tissue, and in lung. Moreover the expression pattern of EDG6 was found to be conserved in mouse and man.

Homology of EDG-6 to the recently identified sphingosine-1-phosphate (SPP) receptors EDG-1, -3 and -5 and lysophosphatidic acid (LPA) receptors EDG-2 and -4, suggests that its ligand may be a lysophospholipid or lysosphingolipid. In collaboration with S. Spiegel, Washington, we have shown that EDG-6 is a high affinity receptor for SPP which couples to a $G_{i/o}$ protein resulting in the activation of growth-related signaling pathways. Although the biological significance of SPP signaling via EDG-6 in lymphocytes and dendritic cells is

poorly understood, the well characterized growth-related or cytoskeleton-associated activities of SPP suggest that members of the EDG family may synergize with signaling pathways initiated by cytokines. Lysosphingophospholipids may play a critical role as potent autocrine and paracrine mediators in specific micro-environmental settings of normal and pathophysiological immune responses.

Differentiation-specific regulation and function of CD155/poliovirus receptor

CD155, a transmembrane protein possessing an Ig-like architecture, was discovered originally by its ability to serve as the cellular receptor for poliovirus (PV). Since then, a lot of effort has been devoted to elucidate the involvement of CD155 in PV infection, a complicated series of events with the potential outcome of the poliomyelitis syndrome in affected individuals. However, there is no

information about the natural function of CD155 and, so, we initiated studies I) to investigate the expression profile of CD155 (by means of analysing the promoter of the CD155 gene) and II) to identify counter receptors/ligands of CD155. Our findings suggest that CD155 is one of the cell adhesion class of molecules since it interacts with vitronectin and, to a lesser extent, with fibronectin. Currently, we are investigating these interactions in more detail. Interestingly, immunohistochemical studies have shown that expression of vitronectin and CD155 colocalizes to the germinal centers of secondary lymphoid tissue.

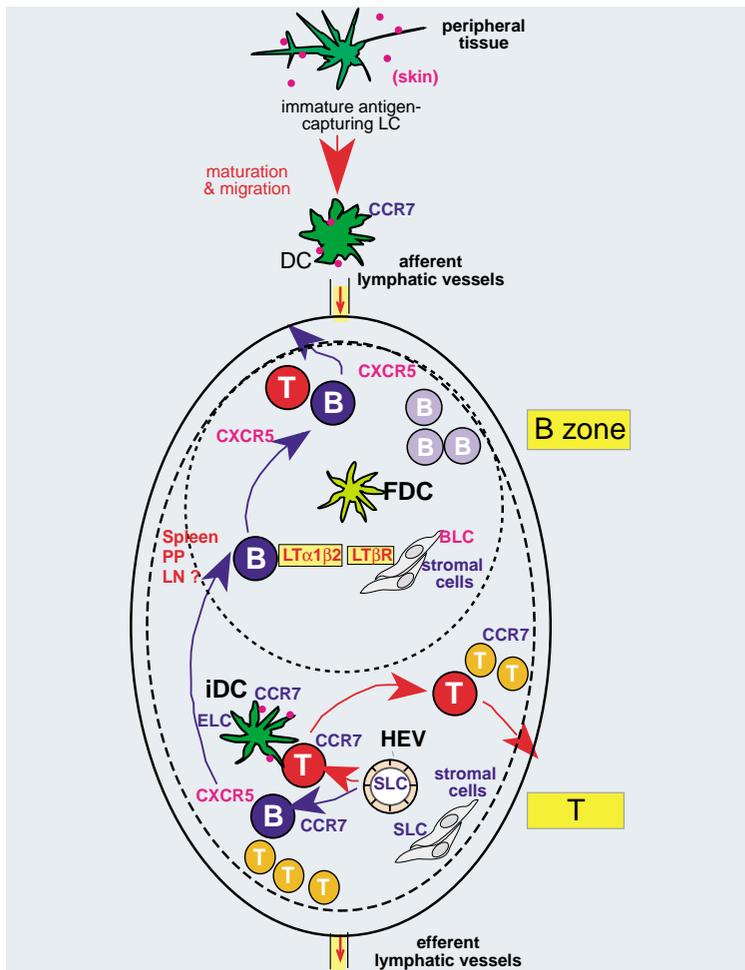


Figure 28: Lessons from chemokine receptor knock outs: Model of chemokine-directed trafficking of lymphocytes and dendritic cells to and through secondary lymphoid organs during the immune response.

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 tumor suppressor protein RB do not only control genes necessary for cell cycle progression, but also induce growth arrest and apoptosis following oncogenic and hyperproliferative signals by activating p53, a tumor suppressor protein known to become 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, we have proposed that functional interaction of E2F and DNA-PK abrogates E2F-dependent transcription and, thereby, congregates the antiproliferative and apoptotic signals induced by DNA-damaging agents.

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Patent Application

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Mechanisms Controlling the 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, both the cis and the trans acting elements in metazoans contributing to the initiation of 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 intend to investigate the architecture of chromosomal replication origins as well as to analyse the proteins binding to them. This work will be 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 like cdc6 and MCM proteins (minichromosome maintenance). Recent data show this interaction also occurs in chorion origins. However, it remains to be determined if this origin is active in cells other than follicle cells and, if so, which of its sequences direct the tissue-specific amplification program. We plan to investigate the former question 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 plan to undertake extensive quantitative expression profiling of the RNA as well as at the protein level. This analysis will be 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, Berlin

Unlike the downregulation of at least some of the replication initiation genes in differentiated cells, reactivation of these genes has to take place in cells which reenter the cell cycle. Thus, one has to postulate that tumor cells, for example, express the complete set of DNA replication initiation genes described above. We want to analyze 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 feasibility 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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Cell Cycle Regulation

Hans-Dieter Royer

Recently, global expression studies have been reported which revealed that hundreds of different genes are specifically expressed in various phases of the cell cycle. Transcriptional control during the cell cycle, however, is poorly understood. Our current work is concentrating on several transcription factors which operate in the G1-, G1-/S- and S-phase. It is the aim of these studies to evaluate the functional role of these factors in cell cycle regulation and human diseases. In these studies, we have discovered that the transcription factor YB-1 is highly deregulated in various malignant diseases, and we have been able to establish a genotype-phenotype correlation for breast cancer. We have shown that YB-1 regulates the expression of the human *mdr-1* gene which encodes P-glycoprotein, a multidrug transporter. Multidrug resistance is a phenotype which seriously hinders chemotherapy, and P-glycoprotein expression is a major factor involved in clinical multidrug resistance. Based on these results, we have started novel projects aimed at understanding the function of YB-1 in malignant diseases, multidrug resistance and cell proliferation. It is the aim of our YB-1 related research to identify signal pathways which lead to nuclear YB-1 accumulation in multidrug resistant tumors and to develop selective inhibitors of this process. With this approach, we intend to develop novel therapeutic strategies for the treatment of clinical multidrug resistance.

Transgenic YB-1 mice

S. Bergmann in cooperation with F. Theuring (Berlin) and M. Dietel (Berlin)

The aim of this project is to analyze the *in vivo* consequences of YB-1 overexpression in breast epithelial cells. We have produced several transgenic mouse lines which express an HA-tagged YB-1 cDNA under the control of the beta-lactoglobulin promoter. The expression levels of YB-1 in these transgenic mice differ considerably. These mice will be monitored over time by histopathological techniques. We are also analyzing YB-1-regulated genes in mouse breast epithelial cells overexpressing YB-1.

Identification of YB-1-interacting proteins

K. Jürchott, Y. Shan, M. Janz in cooperation with R. Kraft (MDC) and J. Behrens (MDC)

The Y-box factor YB-1 is a multifunctional cellular protein which, besides being a transcription factor, participates in several aspects of RNA metabolism. In order to obtain a better understanding of YB-1 functions, we are identifying YB-1 interacting proteins. Cellular YB-1-interacting proteins have been identified by affinity chromatography using a column with YB-1 peptide-antibodies. YB-1-protein complexes were eluted with the immunizing peptide and several novel interacting proteins were identified by amino acid sequence analysis. In addition, we have used the yeast two-hybrid system to isolate several novel YB-1 interacting proteins. We are currently characterizing these interaction partners by GST-pulldown experiments and *in vivo* immunoprecipitation. These studies are aimed at identifying proteins which are involved in regulating YB-1 nuclear transport. The first candidate which could be involved in this process has been identified.

Nuclear overexpression of YB-1 as a prognostic marker for malignant diseases

M. Janz, A. Schmid in cooperation with M. Schmitt, D. Dettmar (München) and M. Dietel (Berlin)

We were the first to discover that nuclear overexpression of YB-1 in human breast cancers is associated with P-glycoprotein expression. Besides being responsible for a multidrug resistant phenotype, P-glycoprotein expression is also a marker for more aggressive tumor behavior. We have initiated several projects which address this issue. One major study has been completed and is currently being analyzed. We have determined the YB-1 expression patterns in over 100 breast cancers whose clinical courses have been determined. This study will show whether YB-1 is a prognostic marker in breast cancer. We are now screening systematically the expression patterns of YB-1 in several human malignancies. These studies will show whether clinical multidrug resistance and deregulated YB-1 expression are also correlated in other malignancies. This work will establish the biological relevance of YB-1 for multidrug resistance and provide a basis for the development of novel therapeutic approaches to the treatment of multidrug resistant tumors.

Development of gene transfer vectors for the treatment of malignant tumors

Ch. Woischwill, M. Janz, K. Jäger in cooperation with G. Wolff and B. Dörken

Overexpression of YB-1 has been observed in breast cancer and certain other malignant diseases. It has been reported that YB-1 is a prognostic marker in osteosarcoma and ovarian cancer. It is the aim of this cooperative project to develop adenoviral gene therapy vectors which contain the YB-1 promoter as a control element for the expression of therapeutic genes. We have cloned the YB-1 promoter and critical promoter elements are being characterized by several approaches. A vector has been constructed containing the complete YB-1 promoter in order to examine transgene expression levels in various mouse tissues and human tumor

transplants. We are now generating therapeutic vectors expressing apoptosis-inducing genes under the control of the YB-1 promoter.

Regulation of human papillomavirus 18 oncogene transcription

E. Grinstein, I. Weinert, W. Jia in cooperation with R. Kraft (MDC), S. Hauptmann and M. Dietel (Berlin)

High risk human papillomaviruses e.g. HPV18 and HPV16 are causative agents of squamous cell carcinomas of the cervix uteri and cervical cancer is the leading cause of death in India and China. Furthermore, the frequencies of cervical cancers are markedly increased in HIV- infected women. In the last two years, we have studied the transcription regulation of HPV18 oncogene expression. The cell cycle-regulated HPV18 enhancer factor p92 was purified and its amino acid sequence has been determined. We have used a p92-GST Fusion protein and have shown that p92 binds in a sequence-specific fashion to the HPV18 enhancer. Antisense knock-out experiments have revealed that p92 is a key regulator of HPV18 oncogene transcription. Using DNase I, we have shown that p92 is involved in regulating chromatin accessibility of the HPV18 enhancer. In addition, we have discovered that, in high grade squamous intraepithelial lesions, p92 expression is deregulated suggesting that *in vivo* deregulated p92 expression levels are a critical event in cervical carcinogenesis. These results have been submitted for publication.

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Weitz, J., Royer, H.-D., Stöhr, M., Kopun, M., and Napierski, I. (1999) Nuclear Inhibitor I-92 and its use for the production of a medicament. *Europäische Patentanmeldung Nr. 92 115 730.1.*

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Epithelial Differentiation, Invasion, and Metastasis

Walter Birchmeier

Our laboratory is concentrating on the molecular analysis of epithelial morphogenesis and differentiation. Earlier, we defined the adhesion and signaling capacities of the E-cadherin/catenin system. Moreover, we have investigated the role of scatter factor/hepatocyte growth factor (SF/HGF) and its receptor, c-met tyrosine kinase, in the morphogenesis of epithelial cells. Epithelial cells can lose 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 or plakoglobin, which mediate interaction of cadherins with the cytoskeleton. We have shown that β -catenin also binds to the transcription factor LEF-1, and that this interaction translocates β -catenin to the cell nucleus and regulates gene expression. 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. One 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. Gab1 is a member of the family of membrane-bound multiadapter proteins which transmit signaling of tyrosine kinase receptors.

Functional interactions of β -catenin with LEF-1, conductin and APC are specified through distinct hot spots in the armadillo domain

Jens-Peter von Kries, Georgia Winbeck, Christian Asbrand, Natalia Sochnikova and Andrea Dell'Oro. In collaboration with Jürgen Behrens (MDC)

β -Catenin is a component of the wnt signalling pathway which plays an important role in developmental processes: wnt signals increase the amount of β -catenin in the cytosol by inactivation of the serine-threonine kinase GSK-3 β . In the absence of wnt signals, GSK-3 β phosphorylates β -catenin which induces ubiquitination and degradation of β -catenin by proteasomes. We have recently found that β -catenin is present in the cytosol as a large multiprotein complex with GSK-3 β conductin or axin, and the tumour suppressor gene product APC. Other labs have reported that mutations in β -catenin or mutations in APC in human tumors stabilise β -catenin and result in constitutive LEF/TCF binding and nuclear signalling. Thus, the regulation of β -catenin stability is pivotal for the transmission of wnt signals in embryonic development and tumor progression. β -Catenin contains a critical sequence, the armadillo repeats 3-8, that provides binding sites for the cytoplasmic fragment of E-cadherin, the 20 and 15 amino acid repeats of APC, the N-terminal region of LEF/TCF and a central domain of conductin/axin.

We have now identified amino acids of β -catenin that directly affect APC, conductin or LEF-1/TCF binding. These residues form separate clusters in the superhelix built by armadillo repeats 3-8. Point mutations in one of the APC or conductin binding sites do not stabilise β -catenin. Mutants unable to interact with both APC and conductin are fully stabilised. So, for degradation, it is sufficient if conductin or APC is recruited indirectly to β -catenin and, thus, to the degradation complex. These mutants will now allow a functional analysis of the individual β -catenin interactions in development or tumor progression.

Requirement for β -catenin in anterior-posterior axis formation in mice

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

The anterior-posterior axis of the mouse embryo becomes explicit morphologically at E6.5, when the first mesoderm forms in the primitive streak region at the posterior side. However, recent experiments show that anterior-posterior polarity is established at least one day earlier: the first signs of anterior-posterior polarity are detectable by asymmetric expression of Cerberus-like, Hex and other markers in the prospective anterior portion of the visceral endoderm. In *Xenopus* and Zebrafish, components of the wnt signaling pathway have been implicated in the induction of embryonic body axis. In *Xenopus*, accumulation of β -catenin on the dorso-anterior side of the embryo is the earliest sign of axis formation. Accordingly, overexpression of β -catenin in *Xenopus* embryos induces formation of an additional embryonic axis.

We recently generated β -catenin-deficient mouse embryos and observed a defect in anterior-posterior axis formation at E5.5, as reflected in the absence of Hex and Hex1 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 show that β -catenin function is essential for anterior-posterior axis formation in the mouse, and experiments with chimeric embryos confirm that this function is required in the embryonic ectoderm.

Coupling of Gab1 to c-Met, Grb2 and downstream effectors mediate biological responses

Ute Schaeper, Martin Sachs, Niels H. Gehring, Renate Franke and Ingrid Walther. In collaboration with Bettina Kemkes (GSF Munich) and Carmen Birchmeier (MDC)

Gab1, like the insulin receptor substrates (IRS), the FGF receptor substrate FRS/SNT, and p62dok family members belongs to a newly identified group of docking proteins that function as specific substrates of tyrosine kinases. Gab1 contains an N-terminal PH domain and a novel phosphotyrosine recognition domain which mediate direct association with the c-Met receptor. Gab1 binds to two sites of the cytoplasmic tail of c-Met, Y14 (Y1349) and to a lesser extent Y15 (Y1356). Gab1 also forms a constitutive complex with Grb2 and this interaction is mediated via the C-terminal SH3 domain of Grb2.

We have now mapped the c-Met and Grb2 interaction sites using reverse yeast two-hybrid technology. The c-Met binding site is localized to a 13 amino acid region unique to Gab1. Insertion of this site into the Gab1-related protein p97/Gab2 was sufficient to confer c-Met binding activity. Association with Grb2 was mapped to two sites: a classical SH3 binding site (PXXP) and a novel Grb2 SH3 consensus binding motif (PP(V/I)(D/N)RXXKP). To detect phosphorylation-dependent interactions of Gab1 with downstream substrates, we have developed a modified yeast two-hybrid assay and identified PI(3)K, Shc, Shp2 and CRKL as interaction partners of Gab1. In a *trk-met* specific branching morphogenesis assay, association of Gab1 with Shp2, but not PI(3)K, CRKL or Shc was essential to induce branching morphogenesis in MDCK cells. A fundamental role of Gab1 for c-Met specific signaling is also supported by gene ablation experiments in the mouse: *Gab1*^{-/-} embryos produced in our laboratory are characterized by strongly reduced and delayed migration of myogenic precursor cells into the limbs, a phenotype reminiscent of *HGF/SF*^{-/-} and *c-Met*^{-/-} mutant embryos.

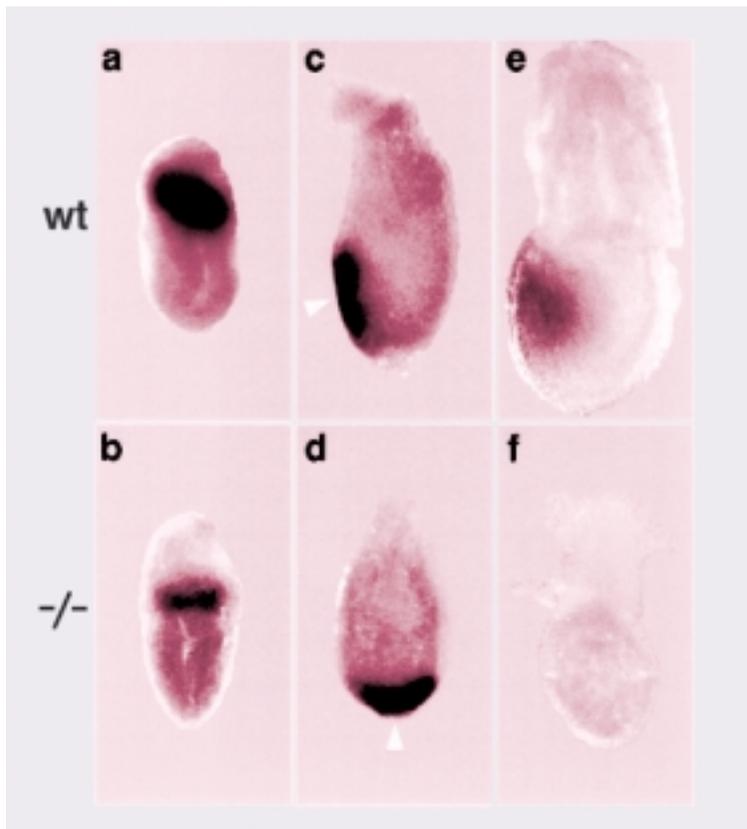


Figure 29: Ablation of the β -catenin gene in mice results in a defect of anterior-posterior axis formation at embryonal day 6.0. The marker *Cerberus* is mislocated in the *-/-* β -catenin embryos (arrow at the distal tip in d) whereas, in the wild-type embryo, *Cerberus* marks the anterior side (arrow in c). Subsequent head development at the anterior side is abrogated (as shown by the marker *Otx2*, compare e and f). Embryonal and extraembryonal tissues are properly developed (as shown by expression of *BMP4*, see a and b). Work by J. Hülken, R. Vogel, V. Brinkmann, B. Erdmann, C. Birchmeier, W. Birchmeier. *J. Cell Biol.* 2000.

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Cell Adhesion and Signal Transduction

Jürgen Behrens

Our group studies the dual role of the cytoplasmic component β -catenin in cadherin-mediated cell adhesion and the wnt signaling pathway. As part of the cell adhesion machinery β -catenin binds to the cytoplasmic domain of cadherins and provides a link to the actin cytoskeleton. In the wnt pathway β -catenin transmits signals to the nucleus by interacting with members of the LEF/TCF family of transcription factors. The wnt pathway controls cellular interactions during embryonal development, and the inappropriate activation of wnt signals leads to cancer. In tumors, mutation of the tumor suppressor gene product APC or of β -catenin lead to the stabilisation of β -catenin and activation of oncogenic target genes by TCF/ β -catenin complexes.

Regulation of β -catenin by conductin in the wnt signal transduction pathway

Boris Jerchow, Martin Würtele, Thomas Schwarz-Romond in cooperation with W. Birchmeier (MDC)

In the past three years, we could identify new components that interact with β -catenin and mediate essential steps in the wnt pathway. In 1996, we identified the functional interaction of β -catenin with the HMG box transcription factor LEF-1. In 1998, we found that β -catenin interacts with the novel protein conductin. We could show that conductin induces the degradation of β -catenin and blocks wnt signaling in *Xenopus* embryos. We could further demonstrate that conductin is a scaffold protein which assembles a multiprotein complex by binding to β -catenin, APC and the serine/threonine kinase GSK3 β at separate domains. GSK3 β is responsible for the phosphorylation of β -catenin which leads to ubiquitination of β -catenin and its subsequent degradation in proteasomes. We propose that conductin coordinates the interplay of β -catenin, APC and GSK3 β in the β -catenin-degradation process by providing a platform for the assembly of these factors. Conductin negatively controls wnt signaling and might act as a tumor suppressor.

We are currently studying the role of conductin *in vivo* by gene ablation in the mouse. We will in particular analyse whether the conductin-negative mice are prone to tumor formation and show lack of control of β -catenin. We also analyse the expression of conductin in tumors and search for possible mutations in the conductin gene (collaboration with P.M. Schlag, Robert-Rössle-Klinik). Further studies aim at the analysis of cellular consequences of conductin-mediated degradation of β -catenin in tumor cells, and the identification of regulators of conductin function by using yeast two hybrid screening methods.

Interaction of β -catenin with SWI/SNF factors

Ralph Wirtz

Transcription of many genes is controlled by a multiprotein complex composed of SWI and SNF proteins, which regulate chromatin assembly by altering nucleosome positioning. We found that β -catenin interacts with a mouse homolog of the yeast SWI3 protein named BAF155. Interestingly, BAF155 and TCF factors associate with different parts of the central arm repeat domain of β -catenin, indicating that β -catenin may provide a link between the two transcriptional regulators. Indeed, in immunoprecipitation experiments LEF-1 co-precipitates with BAF155 which is dependent on the β -catenin binding domain of LEF-1. We propose that β -catenin recruits the SWI/SNF complex to TCFs to promote transcription of wnt target genes. We could also show that SNF5, which is a component of the SWI/SNF complex, competes with β -catenin for binding to BAF155. SNF5 has been assigned a role as a tumor suppressor in early childhood rhabdoid tumors. Mutations of the SNF5 gene lead to C-terminal truncations of the protein. We found that SNF5 mutant proteins show reduced binding to BAF155 and compete less efficiently with β -catenin than wild-type SNF5. We also found that TCF/ β -catenin dependent gene transcription is active in rhabdoid tumor cells and can be blocked by re-introduction of wild-type but not mutant SNF5. We suggest that SNF5 prevents the interaction of the TCF/ β -catenin complexes with the SWI/SNF machinery. The loss of functional SNF5 in rhabdoid tumors may allow recruitment of the SWI/SNF complex to oncogenic wnt target genes.

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Behrens, J., Jerchow, B.-A., Würtele, M., Asbrand, C., Wirtz, R., Grimm, J., Wedlich, D., and Birchmeier, W. (1998) Functional interaction of an axin homolog, conductin, with β -catenin, APC, and GSK3 β . *Science* 280, 596-599.

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Patent Application

“Mittel zur Diagnose und Therapie von Tumorerkrankungen”

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Glycoconjugates

Uwe Karsten

Most cellular proteins require posttranslational modifications in order to function properly. The study of these processes is a necessary complementary approach to research on direct genetic causes of disease.

Glycosylations are among the most common protein modifications. Essential properties of membrane proteins, such as conformation, charge, interaction with other proteins, or biological half-life are largely influenced by glycosylation. The glycans themselves are involved in cell-cell, cell-matrix, and ligand-receptor interactions. Changes in the glycan composition of membrane proteins are regularly found during the course of normal development as well as in cancerogenesis and tumor progression. 'Tumor antigens' are, in fact, often glycotopes and they contribute to the invasive and metastatic growth of cancer cells.

We are studying the structure, distribution, functional role and clinical relevance of cancer-associated carbohydrate antigens, their role in metastasis, and possible intervention strategies. In doing so, we closely cooperate with the Robert Rössle Clinic and others. Special emphasis is given to Thomsen-Friedenreich (TF) and related antigens and their most prominent carrier molecule, epithelial mucin (MUC1). A major goal is the development of novel tumour vaccines against minimal residual cancer based on these antigens.

We are actively involved in international leukocyte and tumor marker workshops (TD-4, HLDA7).

Thomsen-Friedenreich-related antigens and tumor vaccines

U. Karsten, G. Butschak, S. Goletz
Collaboration with H. Gollasch, A. Pezzutto, W. Kemmner, P.M. Schlag (Robert Rössle Clinic), S.E. Baldus, F.-G. Hanisch (Cologne), P. Kristensen (Aarhus), K. Lloyd (New York), S. v. Mensdorff-Pouilly (Amsterdam), H. Paulsen (Hamburg), J. Schreiber (Berlin), H. Shigeoka (Osaka), G. Winter (Cambridge)

In carcinomas the heavily glycosylated, apically expressed high-molecular weight epithelial mucin MUC1 is underglycosylated. This leads to the exposure of otherwise masked peptide epitopes, and to the appearance of new carbohydrate epitopes (TF). In comprehensive studies we have shown that TF is an exceptionally specific tumor antigen, and that its expression in colorectal carcinomas is an independent prognostic marker and risk factor for the development of liver metastases. We have been able to demonstrate in a mouse model that blocking TF epitopes on tumor cells with antibody A78-G/A7 significantly reduces the number of liver metastases. We intend to follow up this finding as a new strategy for the prevention of liver metastasis after resection of TF-positive colon tumors.

So far, tumor vaccines using synthetic MUC1 peptides have been unsuccessful because of their low immunogenicity. We have observed that most MUC1-specific antibodies bind much better if the peptide is glycosylated at the immunodominant PTDR motif with GalNAc or TF. Based on this and other results, we have devised a new MUC1 vaccine with a glycosylated PDTR sequence and a phase I clinical study with this vaccine will start soon.

Carbohydrate antigens, albeit often highly specific tumor antigens, are generally not well suited to vaccine formulations. Their synthesis is expensive and, in most cases, they evoke only incomplete immune responses consisting mainly of IgM antibodies and lacking cytotoxic T cells. To circumvent these drawbacks, we are at present developing carbohydrate vaccines based on molecular mimicry. By employing advanced phage display techniques, among them proteolytic selection, we

have been able to select a number of human single-chain antibody fragments (see figure) and peptides which mimic TF. This is not only the first successful mimicry of the TF disaccharide, but also opens up the hitherto unavailable option of developing DNA vaccines for this and other carbohydrate antigens.

We are also examining the specificities and titers of natural 'anti-TF' antibodies in human sera and their changes in cancer patients in order to explore their potential application as a serum tumour assay.

Carbohydrate-mediated cell adhesion to activated endothelium

R. Stahn, C. Grittner
Collaboration with K. Wenzel (Humboldt University)

Cell adhesion to the vascular endothelium and subsequent extravasation into the surrounding tissue are important steps in inflammatory diseases and cancer. They are regulated by several adhesion molecules and their ligands. E-selectin is crucial for cell 'rolling' on vessel walls as a first step in the adhesion cascade. It is exclusively expressed on activated endothelium, and specifically recognizes carbohydrates of the Lewis type (sLe^x, sLe^a).

We are pursuing two strategies to use E-selectin as a specific target to interfere with the adhesion cascade: 1) blockade of E-selectin binding by means of glycoconjugates carrying sLe^a moieties, and 2) site-specific, E-selectin-mediated transport of agents which interfere with later stages of the adhesion cascade.

Selected Publications

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Patent Applications

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Goletz, S., Karsten, U.: Vakzine gegen konformationsabhängige Antigene. 199 24 405.7; 27.5.99.

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Intracellular Signaling in the Myocardium

Ernst-Georg Krause

The action of the heart as a pump depends on the coordination of electrical, mechanical and metabolic activity of its cells. The prime regulator of these activities appears to be the intracellular calcium concentration which is subject to neural and/or humoral mechanisms. These involve second-messenger mediated phosphorylation of specific proteins which are known to directly influence calcium transport through the sarcolemma and the vesicles of the sarcoplasmic reticulum (SR) in cardiac as well as smooth muscle cells.

Phospholamban, a pentameric protein complex composed of identical subunits, is thought to be the key phosphoprotein in regulating calcium-pump (SERCA) activity of SR vesicles, thereby controlling the relaxation process. In the dephosphorylated state, phospholamban acts as an inhibitor of SERCA, but this inhibition is abolished with increasing phosphorylation. Interestingly, both cAMP-dependent protein kinase (PKA) and Ca²⁺/calmodulin-dependent protein kinase (CaM Kinase) specifically phosphorylate each phospholamban monomer at serine 16 and threonine 17 residues, respectively. The functional significance of this dual-site phosphorylation is not yet known.

In the failing human heart, there is increasing evidence that inotropic stimulation by cAMP-dependent, as well as cAMP-independent, mechanisms results in inadequate diastolic relaxation with consequences for pumping efficiency. These alterations have been suggested to be due to altered intracellular calcium handling. Indeed, a reduced amount of SERCA and an impairment in

phospholamban phosphorylation may lead to a slower diastolic calcium decay. Thus, the function of the SR calcium pump may be modulated at two levels by I) changes in the expression of SERCA, phospholamban and their respective protein kinases and II) alterations of the regulation of calcium transport at the level of SR vesicles.

The research group "Intracellular Signalling in the Myocardium" is engaged in basic research that is mainly focused on molecular mechanisms related to protein phosphorylation and dephosphorylation and to the short-term regulation of contraction and relaxation of the normal and diseased myocardium, as well as long-term processes with regard to Ca²⁺ homeostasis, growth, and differentiation of cardiac muscle cells.

Intracellular signaling in the myocardium

S. Bartel and P. Karczewski in cooperation with R. H. G. Schwinger, University of Köln; M. Kuschel, P. Xiao, E. Lakatta, NIH, Baltimore, USA; A. Kaumann, The Babraham Institute, Cambridge, U. K.; P. Molenaar, University of Melbourne, Australia

We have established that the phosphorylation of phospholamban by PKA at serine-16 prevails over the phosphorylation at threonine-17 and occurs in parallel with the monitored acceleration of relaxation after β -adrenergic stimulation. Interestingly the detectable threonine-17 phosphorylation was inhibited in the presence of the L-type Ca²⁺ channel blocker, verapamil. These data favor a spatial Ca²⁺ signal for activation of CaM kinase activity near the L-type Ca²⁺ channel. Indeed, L-type Ca²⁺ channel activators, like BayK8644 and BayY5959, induce phosphorylation of threonine-17 in the absence of adrenergic stimulation. A dissociation of β -adrenoreceptor signaling from the cAMP regulatory systems has been evaluated for phosphorylation of cytoplasmic proteins, like troponin I and C protein, but not for phosphorylation of the L-type Ca²⁺ channel in the canine heart.

Our data on the short-term regulation by catecholamines in nonfailing and failing human hearts clearly shows

that, in the post-adenylyl cyclase signaling pathway of the failing heart, the phosphorylation of phospholamban is impaired, followed by disturbances in Ca²⁺ sequestration, thereby prolonging the diastolic phase of each cardiac cycle. Elucidating the selective activation of β -adrenergic receptors in the human heart allowed us to clearly show that β -adrenoceptor activation is mediated by activation of PKA and phosphorylation of phospholamban at serine-16 and threonine-17, as well as of troponin I and C protein. These data are relevant to strategies for therapeutic intervention(s) in patients with end-stage heart failure.

CaMKII in heart function and cardiac remodeling

P. Karczewski and B. Hoch in cooperation with J. Bohlender, Franz Volhard Clinic, Humboldt University Berlin; A. Remppis, University of Lübeck; R. Meyer, German Heart Institute Berlin; S. Hatem, INSERM Paris

The multigene family of Ca²⁺/calmodulin-dependent protein kinases II (CaMKII), as a universal mediator in Ca²⁺ signaling, is involved in many of the functions of eukaryotic cells. CaMKII is a multimer consisting of 8 to 12 subunits encoded by four different genes ($\alpha, \beta, \delta, \gamma$). As a major regulator of Ca²⁺ homeostasis, CaMKII is essential for heart function. Despite its importance, little is known about the isoforms of CaMKII expressed in the heart and their specific function in the normal and diseased myocardium. In extension of our previous work, we have characterized four isoforms of the δ -class, the dominant cardiac CaMKII, during rat heart development. Our data have established that the isoform δ_3 is characteristic for the adult myocardium. Furthermore, δ_3 so far characterized as the skeletal muscle isoform, is typically expressed in the embryonic and neonatal rat heart and becomes down-regulated during postnatal development. In hypertensive rat strains, which develop a compensated cardiac hypertrophy, δ_3 is re-expressed whereas the δ_5 transcript levels fall. These alterations are accompanied by changes in the δ -CaMKII protein content in membrane vesicles of the SR.

Depressed contractility in human heart failure has a variety of causes, such as impaired Ca²⁺ cycling, electrical abnormalities and structural remodeling of cardiac cells. We, therefore, characterized δ -isoforms of CaMKII in the human ventricular myocardium. Again, δ_3 is characteristically expressed, whereas isoforms δ_2 and δ_3 are also found in human skeletal muscle. In explanted human hearts with dilated cardiomyopathy, characterized by an increased ANF transcript level and reduced amount of SERCA protein, there was a significant increase in δ_3 transcripts and δ -CaMKII protein. This strongly suggests a role for δ -CaMKII in heart failure with isoform δ_3 being a key determinant.

In the human atrium, the transient outward K⁺ current is important for shaping the action potential and, thus, critical for the development of electrical abnormalities such as arrhythmias. We have obtained evidence that CaMKII regulates voltage-gated K⁺ channels in human atrial myocytes.

Immunocytochemistry of atrial tissue sections shows an intense staining for δ -CaMKII in the intercalated disks, which contain most K⁺ channels. Using immunoblotting, we have demonstrated increased δ -CaMKII expression in tissue specimens from chronically fibrillating human atria. Thus, upregulation of δ -CaMKII could contribute to the electrical remodeling of the diseased atrial myocardium.

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Molecular Muscle Physiology

Ingo L. Morano

The aim of the group is to understand the molecular mechanisms involved in the regulation of cardiac and smooth muscle contraction. Contractility of both cell types is regulated by calcium ions (Ca^{2+}) which penetrate the cells through voltage-gated L-type Ca^{2+} channels and, subsequently, induce the release of large amounts of Ca^{2+} from the sarcoplasmic reticulum into the myoplasm through Calcium Release Channels (Ryanodin Receptors). Ca^{2+} activates the contractile apparatus by binding to troponin C, allowing the molecular motor myosin to interact with thin filaments to produce force and shortening. Due to 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+} channels, Ryanodine Receptors, and Type II myosin in cardiac and smooth muscle. Any structural 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 molecular and functional levels provides the opportunity to develop causal therapies for the treatment of cardiac and smooth muscle dysfunction. We are working with cardiac and smooth muscle preparations from transgenic/knock-out animals and humans as well as cultures of cardiomyocytes and smooth muscle cells.

Understanding the molecular motor

Essential myosin light chain isoforms regulate human heart contractility

Type II myosin isoenzymes are hexamers of about 500 kDa consisting of two heavy chains (MHC) and 4 light chains (MLC), designated essential and regulatory MLC. Two genes coding for cardiac MHC are expressed, β -MHC and α -MHC, located as a tandem on chromosome 14. The cardiac-specific MLC isoforms are designated as atrium- and ventricle-specific essential (ALC-1 and VLC-1, respectively) and regulatory (ALC-2 and VLC-2, respectively) MLC. The cardiomyocytes of hypertrophied ventricles of patients with congenital heart diseases and hypertrophic cardiomyopathy reexpress ALC-1, while MHC isoenzymes are unchanged. This is in sharp contrast to the hypertrophied rodent ventricle which exhibits a change in MHC isoforms rather than MLC expression. The failing ventricles of patients with dilated cardiomyopathy, however, hardly express ALC-1. 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, is increased in hypertrophied human ventricle.

Ventricular cross-bridges with ALC-1 have 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 the Ca^{2+} sensitivity of the force- Ca^{2+} ratio are enhanced.

Besides MHC, essential cardiac MLC isoforms also bind via their N-terminus to actin. Inhibition of the actin-MLC interaction by peptide competition increases force production and shortens the velocity of human heart fibers. We have suggested that interaction between actin and MLC represents a "molecular load" for the cross-bridge, thus depressing its cycling kinetics and force production.

Regulation of smooth muscle contraction by recruitment of non-muscle myosin.

Prolonged smooth muscle activation produces an initial transient state (phase 1) of high maximal shortening velocity (V_{max}) and ATP consumption which is followed by a sustained state (phase 2) of force generation with low V_{max} and ATP consumption. Three different genes coding for MHC are expressed in smooth muscle cells, namely, one smooth-muscle-specific MHC (SM-MHC) and two genes coding for non-muscle MHC. We have mutated the SM-MHC gene by gene targeting technology and found that non-muscle MHC expression remained normal.

Smooth muscle from knock-out (KO) neonatal mice did not exhibit a phase 1, although surprisingly, a phase 2 was observed. Thus, the initial transient phase 1 is generated by SM-MHC recruitment while the sustained contraction state can be generated upon switching from SM- to non-muscle-MHC activation. Non-muscle-MHC-dependent sustained force generation was sufficient for normal fetal development. However, phase 1 *i.e.* high smooth muscle contractility, becomes indispensable for survival and normal growth soon after birth, especially as far as homeostasis and circulation functions are concerned.

Understanding calcium handling proteins

pp700/AHNAK is a cardiac PKA target and binds to the β -subunit of Ca^{2+} channels.

Ca^{2+} channels are multisubunit complexes composed of the pore-forming α_1 subunit along with regulatory β and α_2/δ subunits. Coordinated upregulation of Ca^{2+} channel subunit expression was observed in patients with hypertrophic, but not dilated, cardiomyopathy. Furthermore, we have identified fetal isoforms of both α_2 - and β -subunits. A novel calcium channel-associated protein of 700-kDa was detected in mammalian cardiomyocytes that undergoes substantial protein kinase A phosphorylation (pp700). Amino acid sequence analysis of pp700 revealed homology to AHNAK and pp700/AHNAK is preferentially localized in the plasma membrane of cardiomyocytes. We believe that both phosphorylation of pp700 and its coupling to Ca^{2+} -channels play a physiological role in regulation of cardiac contractility.

Selected Publications

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1998: "New substance to increase cardiac contractility"
PCT/DE98/01240

1999: "A diagnostic marker for the human heart" DPA 199 19 205.7

1999: "Cardiovascular active Peptides" DPA 199 33 090.5

1999: "Drug for the treatment of cardiac insufficiency" DPA 199 38 255.7

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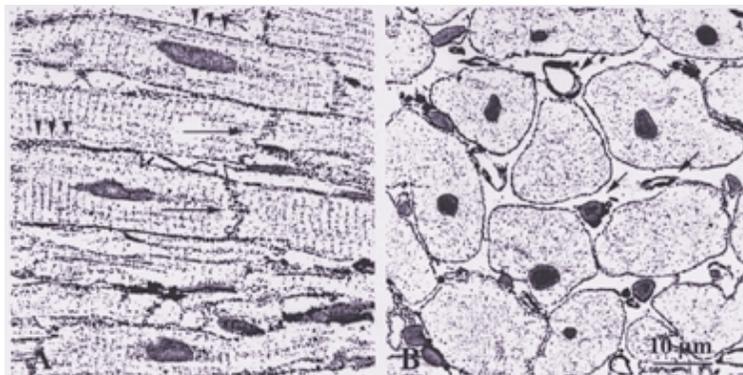


Figure 30: Localization of pp700/AHNAK in rat cardiac tissue (A: longitudinal and B: cross-section). Nuclei are stained with DAPI (in blue). Arrow heads: plasma membrane; large arrows: intercalated discs; small arrows: capillaries (micrograph taken by G. Lutsch, MDC)

Cell Biology of Cardiovascular Diseases

Heinrich Leonhardt
M. Cristina Cardoso

Role and regulation of DNA methylation during development and disease

DNA methylation is essential for mammalian development and has far-reaching effects on gene expression and genome structure. It has been implicated in a number of human illnesses such as Angelman, Beckwith-Wiedeman, and Prader Willi disease and in cancer. In all these cases, functional alleles are shut off by ectopic DNA methylation. Recently, mutations in one of the DNA methyltransferases (Dnmt3b; ICF syndrome) and in a methyl-cytosine binding protein (MeCP2; Rett syndrome) have been implicated in human disease. Methylated cytosine residues are also hot spots for mutations resulting in C to T transitions, the most frequent type of mutation found in human disease.

The longterm goal of this project is to elucidate the regulation of DNA methylation in mammals, i.e. how DNA methylation patterns are changed, how DNA sequences are chosen for methylation or demethylation, how fatal errors in the methylation pattern occur and the role played by genetic and/or environmental factors.

We are addressing these questions via the identification and characterization of functional domains of the known DNA methyltransferases (Dnmt1, 2, 3a and 3b) and searching for interacting factors which might control and direct methylation activity. We have identified a targeting sequence in the regulatory domain of DNA MTase that mediates the association with replication factories and, hence, might warrant the precise maintenance of methylation patterns after each round of DNA replication

(Cell, 71, 865-73). The most dramatic changes in the overall DNA methylation pattern occur during preimplantation development, when 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, hence, might cause demethylation (JCB, 147, 25-32). In addition, we have been able to identify and characterize different isoforms of Dnmt1 (PNAS, 93, 12920-5; JBC, 273, 32757-9). In collaboration Dr. Jaenisch and his group (MIT, Cambridge), we are now studying the role of these functional domains and isoforms in development and disease using transgenic mouse technologies.

Functional organization of the mammalian nucleus and cell cycle control

Several biological processes within the eukaryotic nucleus occur in discrete subnuclear compartments (the most conspicuous being the nucleolus) which, in contrast to cytoplasmic organelles, are not separated by membranes. Different factors involved in a particular process are found concentrated together at the subnuclear sites where the respective processes take place, which is designated "functional organization of the nucleus".

During our analysis of regulatory pathways leading from terminal differentiation to the S-phase, we observed that cyclin A and cdk2 (cyclin-dependent kinase 2) are specifically localized at subnuclear sites of DNA replication and, hence, might function as a link between cell cycle regulation and the control of DNA replication (Cell, 74, 979-992). We could also show that Dnmt1, as well as replication proteins (RPA70, DNA ligase I), are specifically redistributed to nuclear replication foci during the S-phase. Like Dnmt1, DNA ligase I contains a distinct targeting sequence that is necessary and sufficient for association with replication foci. This targeting sequence is dispensable for enzyme activity *in vitro* but is most likely required for the efficient ligation of Okazaki fragments *in vivo* and, hence, may ensure genome integrity in mammalian cells (JCB, 139, 579-587).

Our longterm goal is to study the architecture, assembly and regulation of these replication factories during the cell cycle, including their interaction with cell cycle regulators and other nuclear components.

To study the dynamic regulation of nuclear structures during the cell cycle in real time, we have developed a series of fusion proteins and stable cell lines using a green fluorescent protein (GFP) to visualize these structures in living cells. With these cellular systems, we have been able to 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 within the nucleus. Individual replication foci assemble at a particular nuclear site, keep this position for a given period, and disassemble after finishing DNA replication at this site. Assembly and disassembly of different foci occur asynchronously, suggesting that replication origins also fire asynchronously within these microscopically visible clusters. In collaboration with Dr. Zink and her group (LMU, Munich), we are now labelling subchromosomal domains *in vivo* to study their nuclear localization compared with replication factories and other subnuclear compartments during the cell cycle. In particular, we are investigating whether DNA is reeled through immobile replication factories rather than replication machines sliding down the DNA.

Differentiation and proliferation of smooth muscle cells

The uncontrolled proliferation of vascular smooth muscle cells (VSMC) and the resulting formation of a neointima (called restenosis), after catheter-based therapy including the application of vascular endoprostheses (stents), is currently one of the greatest unsolved problems in interventional cardiology and angiology. This proliferation is mainly due to the ability of SMCs to switch between contractile (differentiated) and synthetic (dedifferentiated) states, where the cells proliferate and migrate to form the neointima.

As far as basic science is concerned, we are investigating the signals and mechanisms controlling the proliferation and differentiation of

VSMCs. We have cloned and characterized an isoform of a cytoskeletal protein (smoothelin) that is specifically expressed in VSMCs (JMM, 77, 294-301). We now intend to use smoothelin as a marker to identify the signal transduction pathway controlling the differentiation of VSMCs.

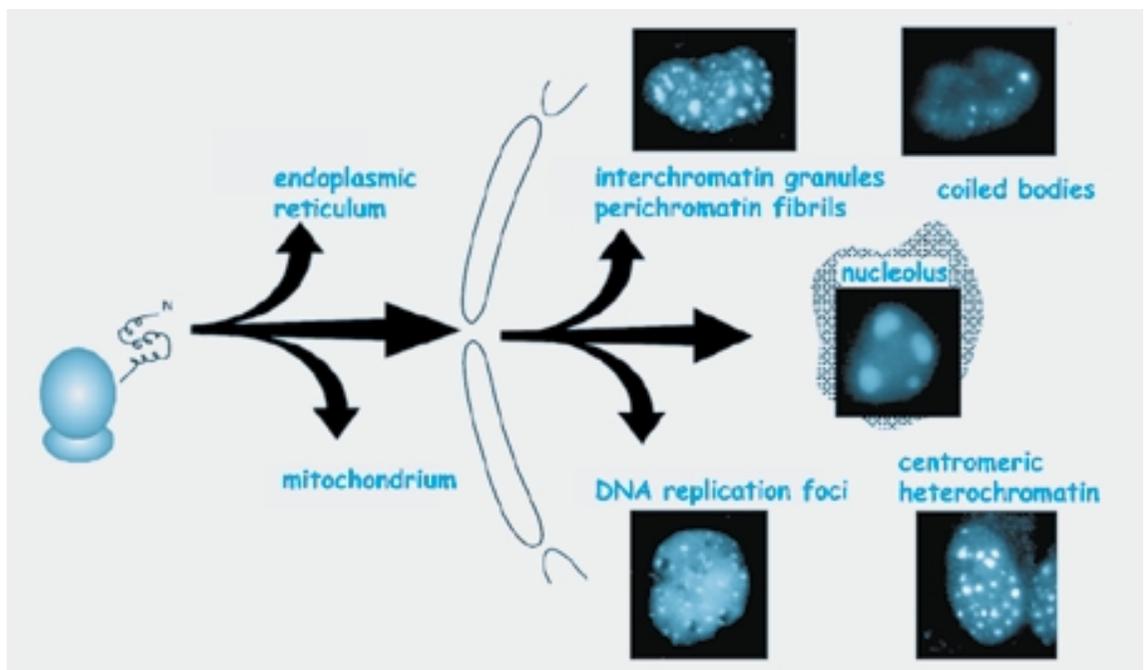
As far as applications of our work are concerned, we are collaborating with Drs. Dietz and Gross (Interventional Cardiology, FVK) and a stent manufacturer to develop new therapeutic options to prevent in-stent restenosis. We are currently testing stents coated with a biodegradable polymer for local drug administration. This approach offers the unique possibility of delivering active substances directly to the diseased and stented segment of the vessel and, thus, to directly influence endothelialization, cell proliferation and migration and matrix deposition.

Terminal differentiation and cell cycle regulation in striated muscle cells

We are currently 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. During terminal differentiation in striated muscle, the level of retinoblastoma protein (pRb) is upregulated whereas cell cycle activators are mostly downregulated and differentiated myocytes are refractile to mitogen stimulation. We have developed cellular systems in which skeletal myotubes can reenter the cell cycle, after either transient expression of viral oncogenes (SV40 T antigen) or deletion of the Rb gene, indicating a central role of pRb in the maintenance of terminal differentiation (Cell, 74, 979-992). In collaboration with Dr. Harsdorf and his group (FVK, Berlin), we are investigating the molecular differences and similarities in terms of cell cycle regulators among the cardiac and skeletal muscle types. We have tested the hypothesis that tumor suppressors like pRb keep cardiac myocytes out of the cell cycle by repressing E2F transcriptional activity, thereby preventing expression of proliferation-associated genes. Indeed, we have been able to induce S-phase reentry in cardiac myocytes using recombinant adenovirus

overexpressing E2F1 in the presence of IGF-1 (Circ. Res., 85, 128-136). Using a cell-free S-phase assay in which isolated nuclei are incubated with extracts from different cell cycle stages, we have further established that, although cardiac myocyte nuclei can be induced to undergo S-phase in the presence of S-phase extracts, an extract from adult cardiac myocytes inhibits S-phase entry (Circ. Res., 85, 294-301). Concomitantly, we are developing new approaches for direct and transient delivery of proteins to affect cellular functions in terminally differentiated cells. We have recently shown that proteins can be directly delivered to differentiated muscle cells by fusion to viral VP22 factor (JMM, 77, 609-613).

Figure 31: Functional organization of the nucleus and subnuclear protein sorting. Upon translation by the ribosomes in the cytoplasm, a protein has several possible fates: it can stay in the cytosol or dependent on having specific signal peptides be imported into different organelles including the nucleus. Within the nucleus, proteins (as well as other molecules) can stay in the nucleoplasm or, although there are no separating membranes, be specifically targeted to one of an ever increasing number of subnuclear compartments. In this diagram we illustrate five of these subnuclear compartments visualized in living mammalian cells by expression of different fusion proteins tagged with the green fluorescent protein.



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Differentiation and Cell Cycle Regulation in Muscle Cells

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Peter M. Schlag

Tumor progression, invasion and metastasis

W. Kemmner, U. Stein, W. Walther, W. Haensch, H. Schwabe in cooperation with U. Karsten, J. Behrens, W. Birchmeier

Tumor-associated alterations of cell surface glycosylation play an important role in invasion and metastasis of carcinoma cells. Accordingly, expression of the glycosyltransferases responsible for glycosylation is altered in tumor cells and this influences their cell adhesion properties. Therefore, sense- and antisense- transfectants of such glycosyltransferases have been established. Specific antisense oligodeoxynucleotides, which inhibit enzyme activity have been studied in connection with a possible clinical treatment of metastasis. Another of our interests are Thomsen-Friedenreich (TF) related tumor-associated carbohydrates. We have found that the balance between O-glycan chain termination through sialylation and chain extension through N-acetylglucosaminylation is disturbed in colorectal carcinomas. Patients with high expression of sialyltransferase ST6GalNAc-II are at a significantly higher risk of death and have a shorter overall survival.

We have identified new genes involved in the organ-specific metastases of colon carcinoma by differential display using surgical specimens. Three ESTs have been identified that are differentially expressed in metastasizing versus non-metastasizing primary tumors, distinct metastases (preferentially liver and lung) or normal epithelium. Expression of candidate genes was confirmed by quantitative real time RT-PCR.

Expression of the transcription factor GLI has been analyzed in sarcomas and during metastasis and we found significantly enhanced GLI expression in tumors compared with normal tissues. Moreover, GLI expression correlated with the grading of the sarcomas. Thus, enhanced GLI expression might be indicative of aggressiveness and dedifferentiation of mesenchymal tumors.

Multidrug resistance and hyperthermia

U. Stein, W. Walther, K. Jürchott in cooperation with B. Rau, P. Hohenberger, P.-U. Tunn

Multidrug resistance (MDR) still limits the successful chemotherapy of cancer. The expression of MDR-associated genes is inducible by external stress factors such as heat shock. This is controlled by stress responsive elements within the gene promoters and might be a molecular mechanism that hinders chemotherapy. Thus, the impact of hyperthermia on the induction of MDR-associated genes has been investigated in colorectal carcinomas, before and after radiochemotherapy or radiochemothermotherapy, respectively. In the majority of the cases analyzed, the risk of inducing MDR gene expression by hyperthermia has been found to be minimal in a clinical setting. Investigations analyzing the influence of hyperthermia on the expression of the MDR genes, 2 – 48h following heat treatment, are currently underway. The impact of isolated hyperthermic limb perfusion on the expression/induction of MDR-associated genes had been examined in soft tissue sarcomas and melanomas at several time points prior to, during and post treatment. So far, induction of certain MDR-associated genes, such as MDR1, MRP1, and LRP, has been observed within hours of hyperthermic treatment. Heat shock-induced expression of MDR genes should be taken into account when combining hyperthermia with MDR-associated cytostatic drugs.

Detection and pathobiology of solid tumors with microsatellite instability

K. Kölbl, B. Barthel, L. Estevéz-Schwarz, K. Krause, H. Pidde, O. M. Ullrich in cooperation with M. Dietel and S. Scherneck

Somatic alterations in simple repetitive sequences that are present as microsatellites throughout the human genome are characteristic of a subset of human tumors with defects in DNA mismatch repair. Germline mutations in various DNA mismatch repair genes dramatically increase an individual's susceptibility to various neoplasms and are the molecular basis for the syndrome of hereditary non-polyposis colorectal carcinoma (HNPCC). HNPCC is characterized by the early and familial occurrence of intestinal and extraintestinal cancers. Collaborative efforts to better define the epidemiological, clinical and pathomorphologic features of this cancer susceptibility syndrome are critical for elucidating its pathogenetic pathways and improving the clinical management of patients and gene carriers. Potential cases of HNPCC, reported locally as well as regionally, have been registered and investigated by a combination of detailed clinical and pedigree studies, histopathology, immunohistology, microsatellite instability (MSI) analysis and germline sequencing. However, identification of HNPCC carriers by biochemical screening prior to complete genomic sequencing could significantly improve these existing cancer preventive strategies. Cell-free DNA in the blood of cancer patients has been shown to harbor microsatellite alterations frequently matching those of the primary tumors. We have investigated the patterns of such microsatellite alterations in sera and microdissected tumors of colorectal cancer patients. Using an integrative morphological, immunohistological and genetic approach, high-grade serum MSI was preferentially found in individuals with mismatch repair-defective tumors. Although the molecular mechanisms of tumoral DNA-shedding remain to be elucidated, its detection by serum DNA microsatellite analysis appears to be useful for the diagnosis and monitoring of neoplasms caused by defective DNA mismatch repair.

The subsequent development of a

malignant phenotype in all solid tumors seems to be determined in part by actin-binding proteins. In earlier studies, our group has shown a regression of the malignant phenotype in MCF7 cells after transfection of the gene of the actin-binding protein profilin. We are now analyzing immunohistologically samples of highly malignant breast tumors in order to select tumors which exhibit defective expression of profilin and, subsequently, analyze the blocked expression mechanism of the profilin gene in these tumors using Western-blotting, quantitative RT-PCR and methylation-specific PCR (MS-PCR).

Non-viral gene transfer for gene therapy of cancer

W. Walther, U. Stein, R. Cartier in cooperation with I. Fichtner and C. Engelmann

Non-viral gene transfer technologies have developed into applicable alternatives to viral delivery systems in gene therapy. A „High-speed Jet-Injection“ system has been tested for gene transfer of naked DNA into tumors. The *in vivo* experiments showed that naked DNA could efficiently be delivered into tumor tissue using the jet-injection technology. The detection of reporter gene expression in jet-injected tumors revealed strong LacZ- or GFP-expression. Therefore, high-speed jet-injection is feasible for an efficient gene transfer into tumors and is applicable to the non-viral gene therapy of cancer. Since nuclear transport of transduced DNA limits the efficiency of non-viral gene transfer, peptides are employed for nuclear targeting of DNA. Peptides harboring nuclear localization sequences (NLS) are complexed with plasmid DNA for improved nuclear transport. Our initial studies indicate the efficient gene transfer of these peptide-DNA complexes, as determined by reporter assays in different tumor cell lines *in vitro*. Confocal microscopy of transduced cells demonstrates the nuclear transport of these peptide-DNA complexes.

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Stein, U., Walther, W. Vector for the expression of therapeutically relevant genes. Patent issued: P 42 38 778 (1995); Germany Patent issued: 5,968,735 (1999); USA.

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Ubiquitin System and Endoplasmic Reticulum

Thomas Sommer

Proteolysis by the ubiquitin-proteasome pathway can be briefly summarized as follows: components of the ubiquitin system recognize proteins destined for proteolysis and covalently conjugate multiple copies of the polypeptide ubiquitin to the substrate. Proteins earmarked in such a way are subsequently recognized by the 26S proteasome which cleaves the proteins to peptides and releases ubiquitin. However, in some cases proteolysis is preceded by an intracellular transport process in which the substrate is presented to the proteolytic system. Currently, two examples of a link between transport and proteolysis are known: ubiquitin-dependent internalization of cell surface proteins and endoplasmic reticulum (ER)-degradation, or ERAD. Our latest results show the existence of a third, and unexpected, linkage of this type.

Protein degradation at the endoplasmic reticulum

Proteolysis of ER-luminal and membrane proteins has been investigated in detail by our group. It is a process common to many eukaryotic organisms and is of significant medical importance. In principle, ER-degradation can be divided into three steps: firstly, an ER-luminal detection system has to be postulated which recognizes misfolded proteins; secondly, the proteolytic substrates are transported back into the cytosol (retrograde transport) via a channel formed by the Sec61p-complex; thirdly, the retrogradely transported substrates are marked with the polypeptide ubiquitin and, subsequently, digested by the cytosolic 26S-proteasome complex.

The ubiquitin-conjugating enzymes, Ubc6p and Ubc7p, the latter of which is anchored to the ER-membrane via Cue1p, are central to this proteolysis. In addition, we have now defined a second pathway of ubiquitin-conjugation involved in ER-degradation (Friedlander et al., manuscript in preparation). Our previous results show that ubiquitination and retrograde transport are tightly coupled, since proteolytic substrates accumulate in the lumen of the ER when ubiquitin-conjugation is abolished. This leads to the hypothesis that ubiquitin-conjugation may contribute to the driving force of retrograde transport. In support of this model, we were able to demonstrate the accumulation of retrogradely transported, ubiquitinated intermediates in the cytosol when we abolished specific functions of the proteasome (Jarosch et al., manuscript in preparation). Apparently, this ER-degradation pathway is conserved during evolution since we recently identified homologs of Ubc6p in higher eukaryotic cells which are involved in ER-degradation of the δ F508 CFTR protein which is found in most cystic fibrosis patients (Lenk et al., manuscript in preparation).

Degradation of nuclear proteins

Recently, we have investigated the turnover of a nuclear substrate, the transcriptional repressor Mat α 2. This approach was based on our previous observation that degradation of Mat α 2p was dependent on Ubc6p/Ubc7p. In summary, our data

suggest that Mat α 2p is degraded via distinct pathways in different cellular compartments. We were able to distinguish a rapid and Cue1p-independent nuclear degradation pathway from a slow and Cue1p-dependent one taking place at the ER-membrane. Thus, a degradation signal only functions in conjunction with its respective cellular localization signal which, in the case of Mat α 2p, is the nuclear localization sequence. In cell biological terms this might represent a mechanism for regulating the half-life of proteins. By transporting a protein into a different cellular compartment, the turnover might be up- or downregulated, probably because the ubiquitination cascades are restricted to certain areas within a eukaryotic cell. This mechanism may be an important tool for regulation, especially for regulatory factors of the nucleus.

Furthermore, we have investigated the influence of nucleo-cytoplasmic transport functions on the proteolysis of the transcriptional repressor and observed that the rapid turnover of Mat α 2p completely relies on nuclear import. Next, we determined the turnover of Mat α 2p when nuclear export was blocked. Intriguingly, we found that the breakdown of Mat α 2p is slowed down by mutations in Cse1p, a karyopherin required for protein export from the nucleus. In addition, we could demonstrate by immunoelectron microscopy that a fusion protein containing the degradation signal of Mat α 2p is transported from the nucleus back into the cytosol. Next we asked whether protein export in general is required or whether the Cse1p pathway is specifically involved. To address this question, we channeled Mat α 2p into a different export route from the nucleus. Such a hybrid protein was rapidly transported out of the nucleus, but this did not result in rapid turnover, indicating that only the Cse1p pathway channels Mat α 2p into rapid proteolysis. Thus, we conclude that Mat α 2p has to be shuttled through the cell nucleus for proper degradation and that rapid proteolysis is linked to Cse1p-dependent protein export from the nucleus.

Our data are consistent with two export models: in one of these Mat α 2 is recognized in the nucleus by a specific ubiquitin ligase that carries a nuclear export signal of the Cse1p pathway. Both Mat α 2p and E3 are exported together and, subsequently, Mat α 2p is ubiquitinated and becomes a target for the proteasome. Consequently, the E3 should also contain a nuclear localization sequence to shuttle back into the nucleus for another round of export and degradation. Alternatively, ubiquitination of Mat α 2p might occur in the nucleus and the export machinery would transport ubiquitin-conjugated Mat α 2p. This transport should involve an adapter protein that binds ubiquitin and carries a nuclear export signal recognized by the Cse1 pathway. Since we do not observe any nuclear export of Mat α 2p from the nucleus in the absence of ubiquitin-conjugation via Ubc6p, we favor the second model. If ubiquitinated proteins were linked to the Cse1p protein export machinery via an adapter protein, all proteolytic substrates could be exported by such an adapter protein. Thus, our model would be applicable to many short-lived regulators of the nucleus. Clearly, further experiments have to be performed to distinguish between these two possibilities.

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- Sommer, T., Jarosch, E., and Lenk, U. (2000) Compartment-specific proteolysis by the ubiquitin-proteasome pathway. *Rev. Physiol. Biochem. Pharmacol.* in press.

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P450 Cytochromes and the Endoplasmic Reticulum

Wolf-Hagen Schunck

In the past two years, our laboratory has begun new projects aimed at elucidating the role of arachidonic acid metabolizing cytochrome P450 enzymes in the regulation of vascular tone and renal function. The major topics of the new projects are human vascular endothelial cells and mouse models of hypertension which are being studied in collaboration with groups at the MDC (V. Gross, B. Erdmann) and Franz Volhard Clinic (H. Haller, F.C. Luft).

Expression of P450 isoforms in human vascular endothelial cells

Epoxy derivatives of arachidonic acid are important autocrine and paracrine mediators in the regulation of a variety of endothelial functions, such as control of vascular tone and inflammation. However, little is known about the molecular identity and regulation of the P450 isoforms actually expressed in endothelial cells which catalyze arachidonic acid epoxygenation and contribute to the different signal transduction pathways involved. To identify potential candidates, we searched for the expression of individual P450 genes belonging to the P450 families 1, 2, 3, and 4. RT-PCR screening performed with subfamily- and isoform-specific primer pairs revealed the presence of mRNAs for the P450 forms 1A1, 1B1, 2C8, 2E1, 2J2, 3A7, 4A11, and 4F2. In addition, P450 1A2 was detected after induction with β -naphthoflavone which also enhanced the expression of P450s 1A1 and 1B1. Similar P450 patterns were obtained analyzing primary endothelial cells originating from aorta, coronary arteries, dermal microvessels, and umbilical veins, as well as an immortalized human endothelial cell line (HMEC-1). HMEC-1 cells were found by gas chromatography/mass spectrometry (GC-MS) to contain a series of regioisomeric epoxyeicosatrienoic acids and to actively produce these metabolites after extracellular addition of arachidonic acid. Among the P450 isoforms detected, P450s 2C8 and 2J2 are leading candidates for producing 11,12-epoxyeicosatrienoic acid, a metabolite recently reported by other researchers to cause vasodilation and have anti-inflammatory properties. Some of the other P450 forms detected may be important under certain pathophysiological conditions (P450s 1A1 and 2E1) or may contribute to eicosanoid degradation (P450s 4A11 and 4F2).

P450-dependent renal arachidonic acid metabolism in normal and hypertensive mice

The starting point for these studies was the physiological data obtained by V. Gross and F.C. Luft showing that hypertension in different mouse models is associated with a reduction in total renal blood flow and a shift to the right of pressure-natriuresis-diuresis curves. These features are typical for desoxycorticosterone (DOCA)-salt hypertensive mice and for angiotensin-type 2 (AT2) receptor knockout mice. Our laboratory became involved in these studies after bezafibrate, an inducer of P450 forms metabolizing fatty acids, was found to improve renal hemodynamics. This finding led to the hypothesis that changes in P450-dependent arachidonic acid metabolism may play an important role in these models. To address this question, we first studied some basic characteristics of mouse renal arachidonic acid metabolism. Using HPLC and GC-MS, the products formed were identified as 20- and 19-hydroxyarachidonic acid (20- and 19-HETE), representing about 80 % and 20 % of the total hydroxylation products. Control microsomes of untreated wild-type mice had arachidonic acid hydroxylase activities of about 200 pmoles/min/mg. Antibody-inhibition experiments indicated the involvement of P450 forms belonging to the 4A subfamily. Induction of hypertension with DOCA-salt resulted in significantly reduced hydroxylase activities which were only about 40 % of the control values. Westernblot analysis revealed that the specific content of P450 4A proteins was markedly reduced. An even more pronounced reduction in the capacity to produce 20-HETE was observed in the kidneys of AT2-receptor knockout mice. Bezafibrate treatment partially restored the low arachidonic acid hydroxylase activities in DOCA-salt mice and, in particular, induced the P450 isoform 4a-14. *In situ* hybridization experiments performed in collaboration with B. Erdmann demonstrated that this P450 is expressed in the cortical-medullary junction where it is predominantly localized in the proximal tubules. Taken together, these results suggest that hypertension in the mouse models studied is associated with a deficiency in the production of 20-HETE by renal tubular structures. Since 20-HETE is known to inhibit

ion channels responsible for salt reabsorption and, thus, stimulate salt excretion, this deficiency provides a reasonable explanation for the observed changes in kidney function. Moreover, we speculate that alterations in tubular 20-HETE production may also affect local renal blood flow assuming transcellular transport and further metabolism of 20-HETE to prostaglandin analogs with vasodilator effects.

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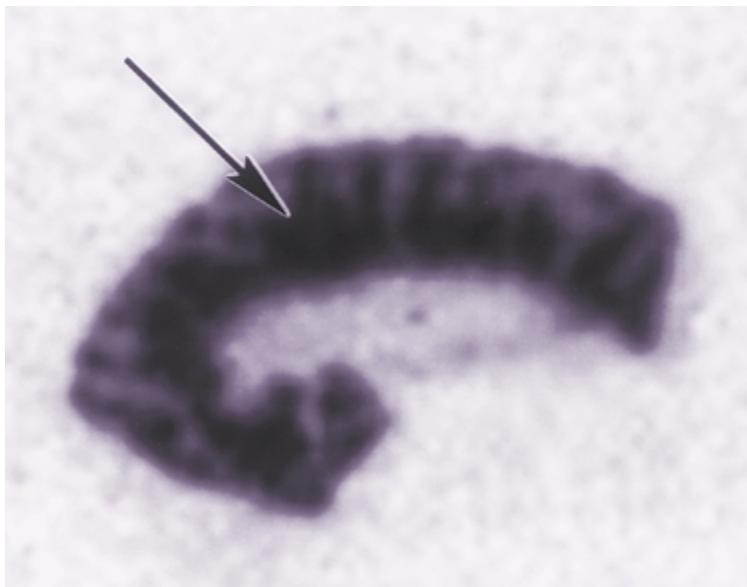


Figure 32: Localization of bezafibrate-induced P450 4a-14 mRNA in the mouse kidney by means of *in situ* hybridization. Phosphor imager picture of a whole kidney section, black staining shows that the P450 4a-14 mRNA is concentrated in the cortical-medullary junction (arrow).

Vascular Biology

Hermann Haller

This group is very interested in the cell biology of the vessel wall. The approaches being used are broad in scope and include patch clamp electrophysiology, signal transduction, cell differentiation and dedifferentiation, gene regulation, and apoptosis. In addition, the group is heavily involved in patient-oriented research directed at elucidating mechanisms relevant to hypertension, including preeclampsia, atherosclerosis, and reperfusion injury.

Endothelial cell function

Endothelial cells and their perturbations (endothelial cell dysfunction) have recently become of major interest in the pathophysiology of chronic vascular disease. Maren Wellner is leading a team that has studied the proliferative effect of vascular endothelial growth factor (VEGF) on human endothelial cells. Two hypotheses have been tested: (1) VEGF affects intracellular calcium regulation and calcium-dependent messenger systems and (2) these mechanisms are important for the proliferative effects of VEGF. Their data show that VEGF induces initial and sustained calcium influx. VEGF leads to translocation of the calcium-sensitive PKC isoform alpha and the atypical PKC isoform zeta. Antisense molecules for these PKC isoforms block VEGF-induced proliferation. These findings suggest that PKC isoforms alpha and zeta are important for the angiogenic effects of VEGF. Maren Wellner has recently directed her attention to signals involved in causing endothelial cells to assume a fenestrated phenotype. Phorbol ester stimulates endothelial cells in this direction and ESM-1 appears to be a marker protein.

Nuclear protein transport pathways

The study of the mechanisms involved in nuclear transport is an exciting area and Matthias Köhler's team is leading this effort. Nuclear proteins, such as transcription factors and ribosomal proteins, are synthesized in the cytoplasm and must be transported into the nucleus to exert their functions. The transport of proteins >20-60 kD through the nuclear pore complex (NPC) into the nucleus is an active, energy-requiring process. Transport substrates are recognized by their transport proteins via certain signals. The best-characterized protein import pathway is the 'classical' nuclear localization signal-dependent pathway with importin alpha and beta carrying the substrate to the NPC. The transport of the importin-substrate complex into the nucleus is regulated by the small GTPase Ran/TC4. More than ten proteins have been discovered which have already been proven, or are very likely, to be nuclear transport factors for distinct import pathways. Members of the importin alpha protein family are very

similar and transport, in a complex with importin beta, nuclear localization signal-bearing proteins into the nucleus. Members of the Ran-binding protein family show a slight degree of similarity to importin beta. Ran-binding proteins share a common domain at the amino terminus which enables them to bind RanGTP, a prerequisite for their function as nuclear import or export factors for distinct proteins or RNAs. Although Köhler has found that Ran/TC4 seems to play a key regulatory role in all nuclear transport pathways, the molecular mechanism of the translocation step through the NPC is still unclear.

Neutrophil apoptosis

Ralf Kettritz is interested in vasculitis, which invariably features neutrophil infiltration and acute inflammation. Recently, he has branched out into neutrophil apoptosis. During inflammation, neutrophils migrate into the affected tissue interacting with extracellular matrix proteins. He has recently tested the hypothesis that neutrophil-matrix interaction affects neutrophil apoptosis. Kettritz has found that the extracellular matrix has a significant effect due to processes regulated by tyrosine phosphorylation. Recently, he performed two-dimensional gel electrophoresis and Western blotting to investigate this. He exposed neutrophils on fibronectin to TNF α and observed several tyrosine phosphorylated proteins, which he subsequently sequenced. One of these proteins was LY-GDI. Ly-GDI cleavage was prevented by caspase-3 inhibition, which also decreased apoptosis. Kettritz has concluded that tyrosine phosphorylation of Ly-GDI, followed by increased caspase-3-mediated Ly-GDI cleavage, is the signaling event associated with accelerated TNF α -mediated apoptosis on fibronectin.

New modes of calcium signaling

Maik Gollasch, Matthias Löhn, and Michael Fürstenau are making exciting advances in electrophysiology. During a Humboldt fellowship at the University of Vermont, Gollasch worked with Mark Nelson and studied local calcium transients termed calcium sparks. These sparks are apparently caused by opening of clustered ryanodine receptors in the sarcoplasmic reticulum. Gollasch's team has investigated caveolae, cholesterol/sphingolipid-rich invaginations of the plasma membrane which colocalize with both the subsarcolemmal occurrence of calcium sparks and the junctional sarcoplasmic reticulum. They have found that a transient elevation in calcium at the inner mouth of a single L-type calcium channel within caveolae induces simultaneous activation and opens several ryanodine receptors to generate a local calcium spark. They are the first to show that a caveolae-calcium signaling pathway may regulate cellular functions via local ryanodine receptors in the sarcoplasmic reticulum. The first illustration of a calcium spark in man (from a coronary artery vascular smooth muscle cell) is shown in the figure.

Christian Maasch, Carsten Lindschau, Gabi Alexander and others are using green fluorescent protein technology to observe protein kinase C α targeting in vascular smooth muscle cells. They recently showed that local changes in calcium regulate PKC α translocation. The targeting mechanism is dependent on the C2 and P domains of the enzyme. Their observations are the first to show that localized calcium changes determine the spatial and temporal targeting of PKC α .

Gene therapy

The group has relied on antisense strategy in a series of exciting studies aimed at treating ischemia-reperfusion injury occurring after organ transplantation and they have used a rat renal transplant model. Duska Dragun has recently completed a study in which she showed that the antisense treatment is very effective in the prevention of ischemia-reperfusion injury in transplantation. A chronic isograft transplantation study has also been completed.

Patient-oriented research

Hermann Haller, Volker Homuth, Friedrich C. Luft, and Gerd Wallukat have collaborated with obstetricians in Berlin to elucidate new mechanisms responsible for endothelial damage in preeclampsia. In a cell culture system of endothelial cell monolayers, the group showed that a factor from the serum of preeclamptic women stimulates increased cell-layer permeability. This process involves PKC signaling, principally PKC α and PKC ϵ . The permeability increase was blocked with antisense against the mRNA of these enzymes. In subsequent studies, the team showed that women with preeclampsia produce agonist autoantibodies directed at the AT1 receptor. The antibodies are directed at the second extracellular loop. Colocalization studies have demonstrated the necessary specificity. The subsequent signaling proved to be a PKC-mediated pathway. Very recently, the group has shown that the antibodies are capable of making vascular smooth muscle cells produce tissue factor. The work of the four investigators was awarded the Galenus von Pergamon prize for 1999.

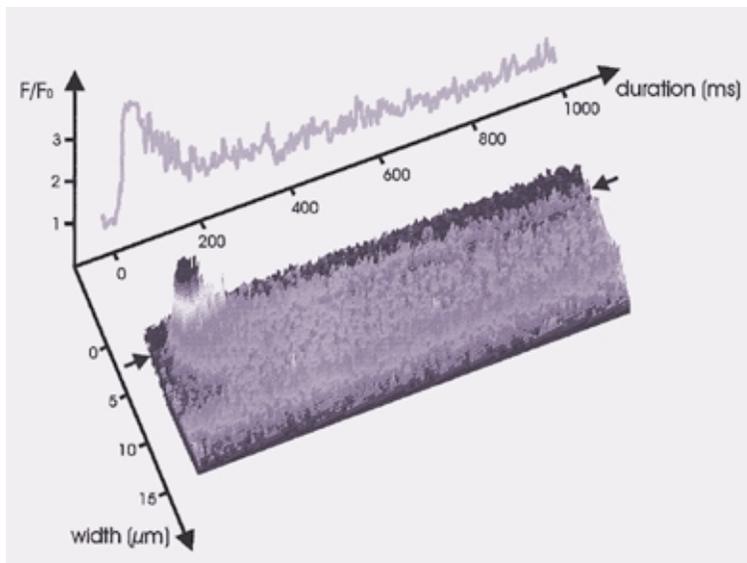


Figure 33: Calcium sparks are localized intracellular events released through ryanodine receptors. The sparks then control excitation-contraction coupling. The confocal line-scan image of a fluo-3-loaded human coronary artery vascular smooth muscle cell is shown. The fluorescence time course of the sparks was determined over the time indicated by the two arrows.

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Functions of Dynamin II and PKC in Post-Golgi Vesicle Formation

Peter Westermann

Cellular functions depend on proper transport and correct subcellular localization of proteins. To accomplish this, secretory proteins, lysosomal proteins and membrane proteins have to be sorted in the trans-Golgi network (TGN) and packed into specific transport vesicles. Vesicle formation is controlled by different G protein families. Therefore, functions of inhibitory trimeric G proteins and of dynamin II have been studied. In addition, the PKC-dependent regulation of vesicle formation at the TGN is being investigated by analyzing Golgi-bound PKC substrates.

New methods for investigating molecular interactions

K. Bulygin, A. Malygin, G. Karpova (Novosibirsk), J. Dong

Two methods have been developed for studying molecular interactions. RNA-DNA interaction have been studied by cross-linking (K. Bulygin et al., 1998) while protein domain interactions have been analyzed by affinity binding of cytosolic or membrane proteins to peptide-tagged protein domains attached to agarose matrices.

Interactions between dynamin II domains and the Golgi apparatus

J. Dong, M. Knoblich in collaboration with A. Otto, E.-C. Müller, and C. Lindschau (FVK)

The functions of dynamins depend on their domain structure. To study the binding of dynamin II to Golgi membranes, the pleckstrin-homology domain (PHD), the proline-rich domain (PRD) and the C-terminal part of dynamin II, consisting of PHD, GTPase activator domain and PRD, were expressed and purified. Interactions between these domains and cytosolic or membrane proteins were studied by affinity binding and cross-linking. PHD binds with high affinity to Golgi membranes, but does not interact with proteins suggesting binding mainly to phospholipids. The proteins that interact with PRD are SH3 domain-containing proteins, amphiphysin I, amphiphysin II and SH3GL2, while additional proteins may bind within larger complexes. The nature and composition of these protein complexes is presently being studied. In addition, membrane-bound, but not cytosolic profilin I promotes attachment of dynamin II to the Golgi apparatus and supports transport vesicle formation (J. Dong et al., in revision).

Identification of PKC substrates attached to the Golgi apparatus

B. Radau, M. Knoblich in collaboration with A. Otto, E.-C. Müller

Stimulation of vesicular transport between the TGN and plasma membrane by activation of PKC (Westermann et al., 1996) may depend on the phosphorylation of Golgi-bound proteins. MARCKS, MacMARCKS, cytokeratin 8, cytokeratin 18 and synaptobrevin 2 have been identified by *in situ* phosphorylation, two-dimensional protein electrophoresis and peptide sequencing as Golgi-bound PKC substrates. The impact of individual phosphoproteins on vesicle formation is under investigation.

Selected Publications

K. Bulygin, K., Malygin, A., Karpova, G., and Westermann, P. (1998) Site-specific modification of 4.5S RNA apical domain by complementary oligodeoxynucleotides carrying an alkylating group. *Eur. J. Biochem.* 251, 175 - 180.

Westermann, P., Knoblich, M., Maier, O., Lindschau C., and Haller, H. (1996) Protein kinase C bound to the Golgi apparatus supports formation of constitutive transport vesicles. *Biochem. J.* 320, 561-568.

Structure of the Group

Group leader

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Guest scientist

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Technical assistant

Maria Knoblich

Electron Microscopy

Members of the electron microscopy group have experience in various microscopic techniques ranging from light microscopy to high resolution electron microscopy. Special importance is given to the application and improvement of immunohisto- and immunocytochemical methods. Recently, methods for correlative immunofluorescence and immunoelectron microscopy have been introduced as well as marked improvements in the preparation of ultrathin cryosections, the most sensitive target for high resolution immunodetection of antigens

Molecular architecture of the nuclear pore complex

M. Vogel, F. Vogel in collaboration with G. Schlenstedt (Homburg/Saar)

To understand the functional role of a particular nucleoporin at the molecular level it is necessary to map its location within the three-dimensional architecture of the nuclear pore complex (NPC). Our new EM sample preparation protocol applied to yeast cells expressing GFP-fused and myc-, Pk- and HA-tagged nucleoporins has enabled the precise localization of a number of transport factors and nucleoporins to distinct structural components of the yeast NPC. According to these results Nup1p, Nup2p, importin α , importin β and exportin (Cse1p) form a new structural and functional complex involved in either nuclear protein import or RNA export.

Compartment-specific proteolysis

M. Vogel, F. Vogel in collaboration with Th. Sommer

The group led by Thomas Sommer has developed an assay to provide evidence for a new and unexpected link between protein export from the nucleus and degradation via the ubiquitin-proteasome pathway (see Th. Sommer's report). We are characterizing this system by EM approaches and are focussing, in particular, on the subcellular localization of different GFP-tagged protein fusions in wild type and export-mutant cells.

Localization of plakoglobin in β -catenin-deficient mouse embryos

B. Erdmann in collaboration with W. Birchmeier, J. Hülsken

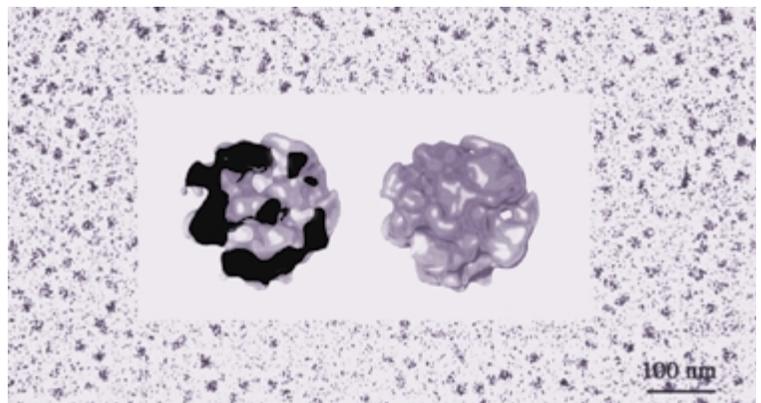
Using a combination of immunofluorescence and immunogold labeling methods, 6-7 days old wild type and β -catenin-deficient mouse embryos could be distinguished and characterized. Following immunocytochemistry of selected areas, an upregulation and redistribution of plakoglobin has been detected along membranes of the mutant embryos.

Structure of small heat shock proteins

G. Lutsch, M. Wieske, R. Wessel in collaboration with J. Behlke, MDC, Berlin, M. Gaestel, Halle, and F. Zemlin, Berlin

Mammalian small heat shock proteins (sHsps) are known to form oligomeric complexes which can act as molecular chaperones. Using electron microscopy, it has been shown that phosphorylation of Hsp25 complexes *in vitro* results in a significant reduction in oligomeric size, accompanied by reduced chaperone activity of the protein. The data provide evidence for regulation of chaperone activity by phosphorylation and dissociation of Hsp25 complexes. Furthermore, cryoelectron microscopy and three-dimensional reconstruction has revealed new details of the 3D structure of ice-embedded Hsp25 complexes which might be of importance for the chaperone function of the protein.

Figure 34: Cryoelectron micrograph of Hsp25 complexes. Inset: 3D structure of Hsp25 complexes. Cropped view (left) and surface view (right).



Localization of smooth muscle myosin heavy chain B and pp700 in cardiac tissue

G. Lutsch, E. Kotitschke in collaboration with H. Haase, I.L. Morano

The 5'-spliced isoform of smooth muscle myosin heavy chain ("intestinal" SM-MHC or SM-MHC-B) and the phosphoprotein pp700/AHNAK have been detected for the first time in cardiac tissue. SM-MHC-B was localized by immunofluorescence microscopy in precapillary arterioles of rat heart, with significantly reduced amounts in ventricles of hypertensive rats, suggesting a role for SM-MHC-B in the regulation of blood perfusion of the heart. pp700/AHNAK was localized to the plasma membrane of cardiomyocytes in accordance with its proposed function in β -adrenergic signal transduction (see report of I.L. Morano).

Further collaborations

Electron microscopic investigations on

- urokinase-induced mitogenesis in human vascular smooth muscle cells (F. Vogel with I. Dumler, D.C. Gulba),
- differentiation-induced changes in antigen uptake mechanisms of avian dendritic cells (F. Vogel with M. Zenke),
- localization of the overexpressed human heart sodium channel protein in EK293 cells (F. Vogel with Th. Zimmer, Jena),
- cellular distribution of generated non-infectious virus-like particles for gene transfer experiments (F. Vogel with R. Ullrich, Berlin),
- influence of different P450 genes on the regulation of ER biogenesis in transfected EK293 cells (F. Vogel with W.-H. Schunck),
- localization of digoxigenin-labeled foreign DNA in endothelial cells following non-viral gene transfer (B. Erdmann with M. Boettger),
- influence of Hsp25 peptides on actin polymerization *in vitro* (G. Lutsch, M. Wieske with R. Benndorf, Ann Arbor), and
- immunofluorescence microscopic investigations on localization of Hsp25 and α B-crystallin in normal and ischemic rat kidney (G. Lutsch with J. Stahl and R. Benndorf, W. Smoyer, Ann Arbor).

Selected Publications

Sandig, G., Kärigel, E., Menzel, R., Vogel, F., Zimmer, T., and Schunck, W.-H. (1999) Regulation of endoplasmic reticulum biogenesis in response to cytochrome P450 overproduction. *Drug Metabol. Rev.* 31, 393-410.

Dube, P., Wieske, M., Stark, H., Schatz, M., Stahl, J., Zemlin, F., Lutsch, G., and van Heel, M. (1998) The rat-liver 80S ribosome at 25Å resolution by electron cryomicroscopy and angular reconstitution. *Structure* 6, 389-399.

Rogalla, T., Ehrnsperger, M., Preville, X., Kotlyarov, A., Lutsch, G., Wieske, M., Arrigo, A.P., Buchner, J., and Gaestel, M. (1999) Regulation of Hsp27 oligomerisation, chaperone function and protective activity against oxidative stress/TNF α by phosphorylation. *J. Biol. Chem.* 274, 18947-18956.

Wetzel, U., Lutsch, G., Haase, H., Ganten, U., and Morano, I. (1998) Expression of smooth muscle heavy chain B in cardiac vessels of normotensive and hypertensive rats. *Circ. Res.* 83, 204-209.

Haase, H., Podzuweit, T., Lutsch, G., Hohaus, A., Kostka, S., Lindschau, C., Kott, M., Kraft, R., and Morano, I. (1999) Signaling from β -adrenoceptor to L-type calcium channel: Identification of a novel cardiac protein kinase A target possessing similarities to AHNAK. *FASEB J.* 13, 2161-2172.

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Dr. Ralf Wessel*

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Martin Wieske*¹

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Erika Kotitschke
Helga Rietzke
Margit Vogel (W.-H. Schunck's group)

* part of the period reported

¹ graduated in 9/98

Molecular Therapy

Molecular Therapy

The aim of this program is to develop new therapeutic strategies for those diseases, such as cancer or cardiovascular diseases, 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 immunogenetic antigens that, nevertheless, fail to elicit an effective immune response from the host. It is also known 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.

The program “Molecular Therapy” consists of the following groups:

Molecular Basis of
Congestive Heart Failure
Prof. Dr. Rainer Dietz

Immunology of
Cardiovascular Diseases
Dr. Gerd Wallukat

Medical Oncology and
Tumor Immunology
Hematology, Oncology and
Tumor Immunology
Prof. Dr. Bernd Dörken

Molecular Immunotherapy
Prof. Dr. Antonio Pezzutto

Molecular and Cell Biology of
Hematopoietic Cells
Dr. Martin Zenke

Phospholipids
Dr. Dietrich Arndt

Drug Targeting
Dr. Regina Reszka

Experimental Pharmacology
Dr. Iduna Fichtner

RNA Chemistry
Dr. Eckart Matthes

Transposition
Dr. Zoltán Ivics
(recently appointed)

Immunology and Gene Therapy
Prof. Dr. Thomas Blankenstein

Hematology, Oncology and Tumor Immunology

Bernd Dörken

Multimarker analysis of cell cycle and apoptosis regulators : definition of novel prognostic factors

Peter T. Daniel, Isrid Sturm, Sandra Herrmann, Alicja Mrozek

Dysfunction of the apoptotic p53/Bax/caspase-3 signaling pathway plays a role in tumorigenesis, tumor progression and development of drug resistance. We are investigating genes and proteins known to be activated in the p53-mediated response to genotoxic damage: BAX, a pro-apoptotic member of the bcl-2 family, and p21, a cyclin-dependent kinase inhibitor, known to mediate the p53-induced G1-arrest. Both are transcriptionally activated by p53, and mutations in the p53 gene may prevent activation of these downstream effectors. Patients with esophageal carcinoma or colorectal cancer with high BAX expression in their tumor lesions have a significantly better survival rate. Multivariate analysis showed that low BAX expression was a highly significant independent negative prognostic marker. In chronic lymphocytic leukemia, the deregulation of p53 or BAX impairs the sensitivity of leukemic cells to cytotoxic drugs. Analysis of the whole signalling pathway, rather than analysis of single genes, such as p53, is crucial and could be useful in predicting the response to cytotoxic therapy. Therapeutic approaches involving transfer of these genes to cancer cells to restore susceptibility to apoptosis are currently being investigated.

Identification of molecular regulators during anti-IgM mediated apoptosis

Kurt Bommert, Anke Rickers, Volker Badock, Niels Peters, Claus Reimertz in cooperation with Brigitte Wittmann-Liebold (MDC)

In order to identify proteins involved in anti-IgM induced apoptosis (which is crucial for elimination of autoreactive B cells) apoptotic and non apoptotic cells of the Burkitt Lymphoma cell line BL 60-2 were compared by high resolution two-dimensional gel electrophoresis and differentially appearing spots were identified by Edman microsequencing and/or peptide mass fingerprinting. The transcription factor SP1 is cleaved into two products of about 68KDa and 45KDa. Using mass spectrometry, we identified a new Caspase-3 cleavage site at position D⁵⁸⁴ leading to a 20kDa protein fragment containing the DNA binding motif, which might act in a dominant negative manner.

Inhibition of Caspase-3 by z-DEVD-fmk inhibits both the cleavage of SP1 and apoptosis, indicating Caspase-3 as a central regulator in anti-IgM induced apoptosis.

We have also identified an early response gene that is strongly upregulated shortly after anti-IgM induction of apoptosis in the BL60-2 cell line but not in the apoptosis resistant subclone R37. The function of these proteins is currently being investigated.

Biology of Hodgkin's disease

Franziska Jundt, Florian Emmerich, Stefan Mathas in cooperation with Claus Scheidereit (MDC)

We recently identified constitutive activation of NF- κ B (p50/p65) as a common feature of Hodgkin/Reed-Sternberg cells which prevents them from undergoing apoptosis and triggers proliferation. To examine possible alterations in the NF- κ B/I κ B system, which might be responsible for constitutive NF- κ B activity, we have analyzed the inhibitor I κ B α in primary and cultured Hodgkin/Reed-Sternberg cells. In the lymph node biopsy of 1 of 10 patients with Hodgkin's disease and in two cell lines (L428 and KM-H2) we detected

mutations in the I κ B α gene, resulting in C-terminally truncated proteins, which are presumably not able to inhibit NF- κ B-DNA binding activity. Our data provide the first indication that constitutive NF- κ B activity in Hodgkin/Reed-Sternberg cells might be the consequence of mutations in the inhibitor genes.

We have also reported that the CC-chemokine eotaxin is strongly expressed in fibroblasts of Hodgkin's disease tissues. Hodgkin/Reed-Sternberg cells induce eotaxin expression in fibroblasts via TNF- α . Our data suggest that eotaxin contributes to eosinophil and T-lymphocyte recruitment in Hodgkin's disease.

Development of gene- and immunotherapy strategies for the treatment of multiple myeloma

Dirk Hönemann, Patric Seibert, Freya Riechert, Kurt Bommert, Ralf Bargou in cooperation with Gert Riethmüller (Munich) and Axel Greiner (Würzburg)

Bone marrow stromal cells (BMSC) produce survival factors that support the growth of multiple myeloma (MM) cells. Interleukin-6 appears to be essential for survival and growth of MM cells. In the absence of BMSCs, dexamethasone, all-trans retinoic acid (ATRA), or the IL-6 receptor antagonist Sant-7 inhibit MM cell growth. If MM cells are co-cultured with primary human BMSCs, they become almost completely resistant to the drugs, suggesting that the bone marrow microenvironment contributes to drug resistance. If dexamethasone and ATRA are given in combination with Sant-7, drug resistance is reversed resulting in almost complete growth inhibition. We are now planning a gene therapy approach for multiple myeloma by stably expressing IL-6 receptor antagonists in BMSCs and hematopoietic stem cells of plasmocytoma patients.

Cytotoxic T cell targeting by bispecific antibodies and chimeric T cell receptors

Anja Löffler, Jan Schwenkenbecher, Ralf Bargou in cooperation with Bernd Groner (Frankfurt), Zelig Eshhar (Israel), and Gert Riethmüller (München)

Cytotoxic lysis by T cells requires specific binding of the T-cell receptor complex to antigenic peptides presented by MHC molecules. Bispecific antibodies can bypass this requirement by targeting T lymphocytes to cells that express an antigen recognized by a monoclonal antibody. Using a recombinant bispecific single-chain antibody (CD19 x CD3) we have been able to induce rapid and highly effective lymphoma cell cytotoxicity by unstimulated T cells. In collaboration with G. Riethmüller we are planning a phase I study for the treatment of lymphoma patients. Currently, we are trying to establish the same strategy for the treatment of multiple myeloma patients using a novel plasma cell-specific surface antigen as a target structure. Tumor cell-targeting can also be achieved by chimeric T-cell receptors, whereby the cytoplasmic part of the ζ -chain of the T-cell receptor/CD3 complex is fused to a recombinant single chain antibody. Using retroviral vectors, we have achieved high transfer rates and stable surface expression of chimeric T-cell receptors with different specificities in T cells. A clinical phase-I trial in patients with metastatic breast cancer is planned.

Cell-biologic features of acute leukemias

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

Acute lymphoblastic (ALL) and acute myeloid leukemias (AML) exhibit a high degree of genotypic diversity. Recently, significant associations between immunophenotypic and genotypic features have been described that in the near future might contribute to the development of individually adjusted treatment strategies. In the last few years, we have characterized the expression and function of molecules involved in apoptosis regulation and chemosensitivity modulation in

T-lineage ALL and AML subtypes. Spontaneous apoptosis, cytokine responsiveness and expression of apoptosis-regulating Bcl-2 and Bax proteins have been analyzed in leukemic blasts from T-ALL patients. IL-7, in contrast to IL-4 and IL-2, is a highly efficient inhibitor of apoptosis and this correlates with the expression levels of the IL-7 receptor (α -chain as well as upregulation of Bcl-2 protein expression. In a large series of T-ALL samples (n=130), *in vitro* IL-7 responsiveness is associated with cortical/mature immunophenotype and better *in vivo* early cytoreduction. This suggests that IL-7 responsiveness might have potential prognostic relevance as a surrogate marker reflecting differential survival factor dependence, apoptosis regulation and treatment response in T lineage ALL.

Resistance to chemotherapy-induced apoptosis and multiple-drug-resistance (MDR) activity, mainly mediated by the efflux pump P-glycoprotein (P-gp), contribute to the failure of chemotherapy in hematologic malignancies. In a large series of adult de novo AML patients, the most immature AML cells exhibited a significantly lower CD95 (Fas/APO-1) expression, CD95 sensitivity and extent of spontaneous apoptosis *in vitro* as well as a significantly higher Bcl-2 expression and P-gp function, compared with more mature AML blasts. Several functional parameters, including high P-gp function, low spontaneous apoptosis *in vitro*, high Bcl-2 expression and low CD95 sensitivity, have been found to be predictive of a poor response to induction chemotherapy in adult de novo AML. Prospective studies monitoring apoptosis-related parameters during chemotherapy in cytogenetically defined risk groups are in progress.

Selected Publications

Sturm, I., Köhne, C.H., Wolff, G., Petrowsky, H., Hillebrand, T., Hauptmann S., Lorenz M., Dörken, B., and Daniel, P.T. (1999) Analysis of the p53/BAX pathway in colorectal cancer: low BAX is a negative prognostic factor in patients with resected liver metastases. *J. Clin. Oncol.* 17, 1364-1374.

Rickers, A., Beyaert, R., Vandenabeele, P., Dörken, B., and Bommert, K. (1999). Cleavage of transcription factor SP1 by caspases during anti-IgM-induced B Cell apoptosis. *Eur. J. Biochem.* 261, 269-274.

Löffler, A., Kufer, P., Lutterbüse, R., Zettl, F., Daniel, P.T., Schwenkenbecher, J.M., Riethmüller, G., Dörken, B., and Bargou, R. (2000) A recombinant bispecific single chain antibody CD19xCD3 induces rapid and high lymphoma directed T cell cytotoxicity. *Blood*, in press.

Jundt, F., Anagnostopoulos, I., Bommert, K., Emmerich, F., Müller, G., Foss, H.-D., Royer, H.-D., Stein, H., and Dörken B. (1999) Hodgkin/Reed-Sternberg cells induce fibroblasts to secrete eotaxin, a potent chemoattractant for T cells and eosinophils. *Blood* 94, 2065-2071.

Emmerich, F., Meiser, M., Hummel, M., Demel, G., Foss, H.-D., Jundt, F., Mathas, S., Krappmann, D., Scheidereit, C., Stein, H., and Dörken, B. (1999) Overexpression of I kappa B alpha without inhibition of NF- κ B activity and mutations in the I kappa B alpha gene in Reed-Sternberg cells. *Blood* 94, 3129-3134.

Ludwig, W.D., Rieder, H., Bartram, C.R., Heinze, B., Schwartz, S., Gassmann, W., Löffler, H., Hossfeld, D., Heil, G., Handt, S., Heyll, A., Diedrich, H., Fischer, K., Weiss, A., Völkers, B., Aydemir, Ü., Fonatsch, C., Gökbuget, N., Thiel, E., and Hoelzer, D. (1998) Immunophenotypic and genotypic features, clinical characteristics, and treatment outcome of adult Pro-B acute lymphoblastic leukemia: Results of the german multicenter trials GMALL 03/87 and 04/89. *Blood* 92, 1898-1909.

Structure of the Group

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Molecular Immunotherapy

Antonio Pezzutto

Our group is focusing on the development of molecularly defined immunotherapy strategies for the treatment of renal cell carcinoma, colorectal carcinoma and chronic myeloid leukemia. A gene-modified tumor cell vaccine, that expresses a shared renal carcinoma antigen recognized by T cells in the context of HLA-A*0201 (developed in cooperation with Th. Blankenstein (MDC) and D. Schendel (GSF, Munich), is being developed in our GMP laboratory in the clinic: the first patients will be recruited in Spring 2000. Other approaches in renal cancer include cytokine administration studies and vaccination protocols using dendritic cells.

Induction of T-cell immunity against EpCam (Epithelial Cell Adhesion Molecule)

Günther Richter, Frank Kaiser

We have found that some patients with colorectal cancer develop an MHC-II restricted response against peptides of the epithelial adhesion molecule EpCam, which is overexpressed in several human adenocarcinomas. The EpCam-specific monoclonal antibody CO17-1A (Panorex®) is used in the adjuvant treatment of colon cancer, T-cell immunity seems to contribute to this therapeutic activity. A correlation between the presence of EpCam-specific T cells and the clinical course is currently being investigated. Transgenic mice expressing human EpCam and human HLA-A2 are being generated for use in vaccination experiments in order to evaluate the safety and toxicity of EpCam-directed immunity. Dendritic cells (DC) pulsed with recombinant EpCam protein or selected EpCam MMC-I and MMC II peptides, gene-modified EpCam-expressing DC or EpCam-Adenovirus, will be evaluated for their ability to induce rejection of EpCam-positive tumors. The feasibility of a clinical vaccination study in patients with EpCam-positive adenocarcinomas will be evaluated.

Use of dendritic cells for the induction of antileukemic immune response

Monika Schwarz, Günther Richter, Jörg Westermann, Kang Hun Lee

Nonamer 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. Indeed, HLA-B8 and HLA-A3 appear to protect against the development of CML as shown in recent epidemiology studies. 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 appears to be safe and flexible. Evaluation of the immune responses is ongoing. In cooperation with B. Wittman-Liebold, A. Otto, and B. Wittmann (MDC, protein biochemistry), 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, and an assay based on the use of HLA-Class I tetramers 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.

Gene modification of dendritic cells

Jörg Westermann, Tam Nguyen-Hoay, Andreas Molweide

Both human and murine DC can be gene-modified using retroviral vectors and receptor mediated endocytosis (targetting the mannose receptor). Complexes of DNA, polyethylenimine (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 the 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 an altered immune response with prevalence 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.

Selected Publications

Westermann, J., Aicher, A., Qin, Z., Cayeux, S., Daemen, K., Blankenstein, T., Dörken, B., and Pezzutto, A. (1998) Retroviral interleukin-7 transduction into human dendritic cells: a strategy to increase T-cell activation. *Gene Therapy* 5, 264-271.

Daniel, P.T., Kroidl, A., Kopp, J., Sturm, I., Köhne, C.H., Moldenhauer, G., Dörken, B., and Pezzutto, A. (1998) Immunotherapy of B cell lymphoma with CD3xCD19 bispecific antibodies: costimulation with CD28 prevents "veto" apoptosis of antibody-targeted cytotoxic T cells. *Blood* 92, 4750-4757.

Cayeux, S., Richter, G., Becker, C., Pezzutto, A., Dörken, T., and Blankenstein T. (1999) Direct and indirect T cell priming by dendritic cell vaccines. *Eur. J. Immunol.* 29, 225-234.

Fan, W., Richter, G., Cereseto, A., Beadling, C., and Smith, K.A. (1999) Cytokine response gene 6 induces p21 and regulates both cell growth and arrest. *Oncogene* 18, 6573-6582.

Aicher, A., Shu, G., Magaletti, D., Mulvania, T., Pezzutto, A., Craxton, A., and Clark, E.A. (1999) Differential role for p38 MAPK in regulating CD40-induced gene expression in dendritic cells and B cells. *J. Immunol.* 163, 5786-5795.

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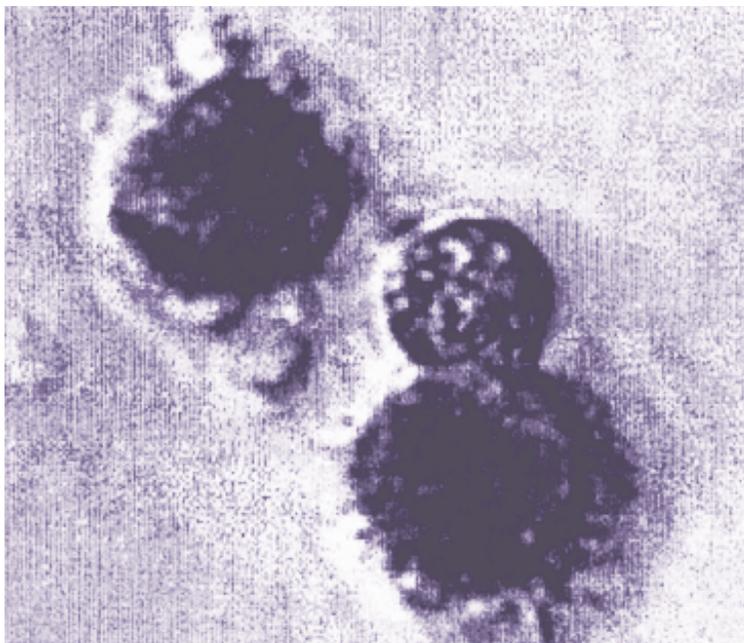


Figure 35: Two gene-modified DC expressing the β -Galactosidase reporter gene (a nuclear localisation signal determines the darker staining of the nucleus) close to a normal, non transduced lymphocyte.

Experimental Pharmacology

Iduna Fichtner

The group is continuing with the development of *in vitro* and *in vivo* models of relevance to preclinical investigations in cancer. With the help of these models, we shall address questions concerning :

- the expression of tumor markers in relation to biological characteristics, such as metastasis, drug- or hormone resistance,
- the pharmacological potential of novel therapeutic or diagnostic approaches, or
- the possibility of mitigating therapy-induced side-effects.

Expression of tumor-related markers

In this research, the occurrence of markers for metastasis (CD44), resistance (MDR), endocrine dependence (estrogen receptor) or immune defence (RANTES) have been correlated with the properties of tumor growth.

The surface marker CD44 and several of its splice variants are expressed in a very specific pattern in individual breast cancer xenografts, as revealed by RT-PCR and immunohistochemistry. The detection of certain CD44-isoforms is not related to the hormone dependence or metastasis capacity of the tumors. Cytostatics and antihormones used clinically for the treatment of breast cancer do not affect the expression pattern of CD44 in xenografts indicating that it is a suitable target molecule for gene- or immunotherapeutic approaches.

In a clinically related study in 14 human sarcomas, we have found a close relationship between the expression of the multidrug resistance gene (*mdr1*) and the response to doxorubicin, both in xenografts and patient tumors, while for lung resistance protein (LRP) and MDR-associated protein (MRP) there was only a poor correlation. We have concluded that screening sarcomas for MDR-related markers clearly predicts chemoresistance and helps avoid unnecessary and toxic treatment.

In cooperation with the University of Mannheim, the chemokine RANTES has been found to be expressed by a subset of melanomas. It is responsible for the recruitment of monocytes, T-cells and dendritic cells but, surprisingly, it favored tumor formation in nude mice.

Models for novel therapeutic or diagnostic approaches

In cooperation with the Department of Pediatric Oncology/Hematology of the Virchow-Clinics leukemic blasts of patients have been transplanted to severely immunodeficient NOD/SCID mice. In all, 11/16 acute lymphatic leukemias were successfully established *in vivo* and shown to maintain their immuno- and genotype during passaging. The antileukemic activity of allogeneic human mononuclear cells as a graft versus

leukemia (GVL) reaction with an accompanying graft versus host disease (GVHD) was simulated in the mouse model. The chemo- and radiation sensitivity of the ALL lines resembled that in a clinical situation. We believe that xenotransplanted ALL can be considered as clinically relevant models mimicking human conditions and are a useful preclinical tool for the evaluation of novel immuno- or gene therapeutic approaches.

Another extended study deals with the detection of minimal residual disease (MRD) in the bone marrow of patients with solid tumors. At present, occult epithelial cells are determined by immunohistochemical or PCR methods in patient samples. However, nothing is known about the viability of these cells or their proliferating and metastasizing potential. Therefore, bone marrow samples of 13 patients with breast cancer, 30 from colorectal cancer (Robert-Rössle-Clinics) and 33 from head and neck cancers (Mund-Kiefer-Gesichtschirurgie, Virchow Clinics) were transplanted to NOD/SCID mice. Human and epithelial cell-specific detection methods have been developed for a sensitive proof of potential cancer cells in murine organs. The results obtained show that only in rare instances can vital cancer cells be found. These findings correlate with the poor prognosis for the disease. Additionally, the results suggest that the evidence of epithelial cells in bone marrow samples results in too many false positives concerning the survival potential of those cells.

Engraftment of non-hematopoietic progenitor cells from human blood in immunodeficient mice

Human cord blood (CB) and human mobilized peripheral blood (PB) are attractive cell sources for hematopoietic transplantation, but their potential to form non-hematopoietic cells is as yet poorly characterized. Six to nine weeks after injection of separated CD34⁺-cells from CB and PB into sublethally irradiated NOD/SCID-mice we found besides human hematopoietic cells (up to 40 %) in chimeric bone marrow, cells staining positive with antibodies specific for human fibroblasts and human endothelial cells. PB CD34⁺-cells were flow-cytometrically sorted into CD34⁺/CD38^{low} and CD34⁺/CD38^{high}-

fractions. The hematopoietic potential was found predominantly in the CD34+/CD38^{low} -fraction, while human fibroblasts marker-positive cells and human endothelial cells were much more commonly detected after transplantation of the CD34+/CD38^{high} -fraction. These data show that non-hematopoietic cell populations are present in human blood cell transplants, engraft *in vivo* and probably support donor hematopoiesis. This technique provides a preclinical model to evaluate clinical protocols involving the transplantation of hematopoiesis-supporting stromal populations into patients with myelotoxic and myelodysplastic disorders.

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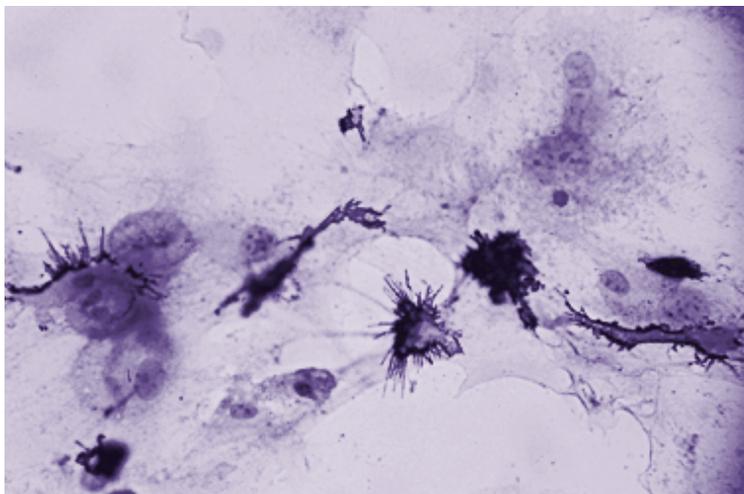


Figure 36: Detection of human endothelial cells (EN4-positive) in long term cultures of chimeric bone marrow derived eight weeks after transplantation of separated cord blood CD34+ cells in NOD/SCID mice.

Drug Targeting

Regina Reszka

The major focus of our group is the development, characterisation, and testing of new drug carrier systems for both therapeutic and diagnostic applications. Therapeutic approaches include the establishment and optimisation of *in vivo* liposomal gene transfer of “suicide” genes, human chemokine genes, as well as p53-independent apoptosis-inducing genes all for the treatment of primary brain tumors and liver metastases. With regard to immunological gene therapy, we are investigating the use of human chemokine genes to attract leukocytes and to modulate the angiogenesis of different tumors.

Therapy of liver metastases

One strategy in anticancer gene therapy is the use of “suicide” genes. We have concentrated on the tumor-specific expression of *Herpes simplex* virus thymidine kinase gene (HSV-tk) expression under the control of the carcino-embryo-antigen promoter (CEA).

In contrast to viral-based strategies, our delivery approach uses *in vivo* cationic and surface-modified liposomal gene transfer systems administered intrahepatically. For the effective transfer of marker (figure) 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 efficiency.

Therapy of glioblastomas

For the *in vivo* transfection of rat glioblastomas with the TK suicide gene, we are using three different cationic liposomal formulations, including our own DAC-CHOL/DOPE, to deliver the pUT TK vector. The liposomal delivery of pUT TK has been compared with adenoviral and retrovirus producing systems carrying the HSVtk gene. Although none of these systems is capable of efficient gene delivery *in vivo* following a single application, a key advantage of the liposomal system is that we can administer the liposome-DNA complex continuously over three days via a mini-osmotic pump. With this technique, we can obtain significant tumor regression. The assessment of the safety and toxicity of this gene delivery show no organ pathology. We have demonstrated by immunohistochemistry that only animals with complete tumor rejection exhibit macrophages as well as T- and B-lymphocyte infiltration in the former tumor area. This suggests an immune host response following therapy and supports the hypothesis that this effect is necessary for therapeutic success.

Our present *in vivo* studies are focused on optimising the administration route and schedule of ganciclovir dosing. The non-viral suicide gene delivery system, using either continuous i.v. infusion or direct CNS application *via* pumps, is

now being introduced in a clinical trial involving two neurosurgery centers, in Cologne and Düsseldorf.

An additional therapeutic approach is the evaluation of the biological effects of selected rat chemokines on rat brain tumor development. F98 cells have been stably transfected with the sub-cloned rat chemokines MIP-1 α , MCP-1 and Mob-1 and inoculated in rat brains. Inoculated non-transfected F98 cells serve as a control. The biological activities of MIP-1 α , MCP-1 and Mob-1 have been evaluated with regard to tumor growth, immune response and microglial activation.

We are currently investigating various newly synthesized biodegradable cationic lipids, with cholesterol or glycerol backbones, as improved gene delivery systems.

Another interest is the characterization of two novel gene transfer systems from Quiagen, SuperFectTM and EffecteneTM. SuperFectTM is an activated dendrimer and EffecteneTM a novel cationic lipid with an additional DNA-condensing agent. The aim of these studies is the characterisation of the electrostatic and colloidal properties which give rise to effective gene transfer. These data will provide a deeper insight into the structure-activity relationships of gene vectors.

To further advance the gene therapy for cancer, we are cooperating with Prof. Winter's group in Dortmund to develop an implantable drug depot encapsulating clinically well established cytostatics with known dose-limiting toxicity, such as Carboplatin and Taxol. This novel system represents a cubic phase structure which releases both encapsulated drugs with different sustained pharmacokinetics. It will be used for the local chemo-treatment of glioblastomas after surgery. To gain more genetic and basic molecular information about the growth characterisation and invasiveness of recurrent glioblastoma, we are cooperating with the neurosurgery unit of Berlin-Buch to develop and expand a brain tumor bank. This resource includes an extensive catalogue of CNS tumors, including different stages of glioblastoma and astrocytoma (grade II, III, IV).

Interaction of pharmacologically active substances and different types of drug carriers

To obtain a detailed knowledge of the molecular regulation of phospholipid asymmetry in tumor cells after treatment with pharmacologically active substances, as an indicator of apoptosis or growth regulation, we have studied calcium signaling processes in different cell lines.

Two dimensional polyacrylamide gel electrophoresis (2-D PAGE) has been established to determine the plasma protein adsorption patterns of liposomes as a determinant for organ distribution. Information about the correlation between protein adsorption and *in vivo* organ distribution can be used to achieve drug delivery to the desired target sites.

For the pulmonary administration of liposomes, we have developed a new technique to generate a liposomal dry powder aerosol by spray evaporation. Using this mild method, the particle size of the aerosol can be adjusted to the requirements of the particular disease, resulting in an improved deposition rate and, consequently, better

therapeutic effect with reduced side-effects. Furthermore, a new targeting concept will be established based on protein-receptor interactions to increase drug concentrations in the target tissue.

New magnetic particles could be isolated and processed from the bacterium, *Magnetospirillum gryphiswaldense*. These so-called "magnetosomes" are now being developed as NMR-diagnostic and therapeutic formulations and as conventional and gene-transfer tools.

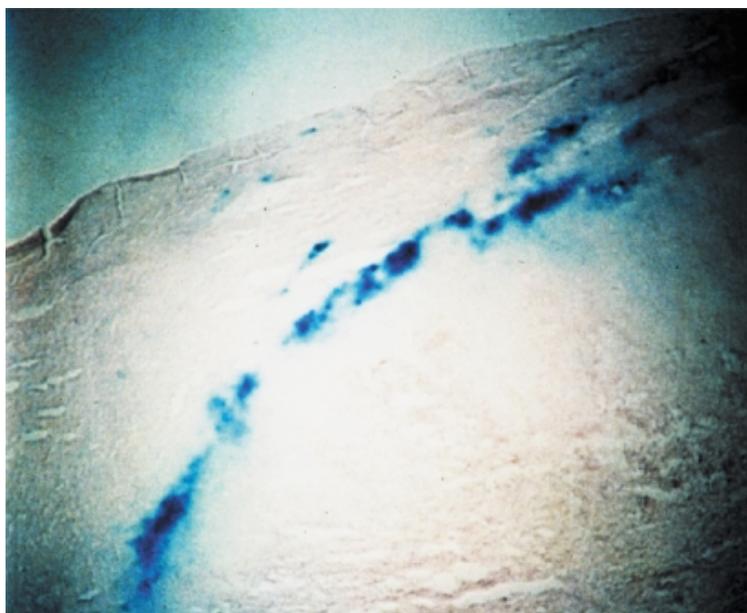


Figure 37: Lac Z expression in the marginal liver tumor zone in rats after a single application of 10 μ g LacZ gene (pUT 651) (Treatment schedule: 10⁵ CC 531 cells were inoculated directly subcapsularly into the liver of male Wag/Rij rats at day 0, treatment was at day 10 with the LacZ DCES, and rats were sacrificed at day 15, histochemistry of the tissue sections).

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submitted: 3.2.1999
Reference: 199 05 285.9-41

Reszka, R., and Schlüter R. Implantierbares Wirkstoffdepot
submitted: 6.8.1999
Reference: 199 38 331.6

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Molecular Basis of Congestive Heart Failure

Rainer Dietz

Regulation of cardiac cell growth and death

Frequently, congestive heart failure is the clinical consequence of a structural remodeling of the cardiac phenotype, which is characterized by two major components: maladaptive growth and excessive death of cardiomyocytes. Therefore, in order to understand the molecular basis of congestive heart failure one has to elucidate the signaling cascade controlling both the growth and death of cardiac cells and describe their interrelationship.

In contrast to cardiac growth where numerous stimulating factors have been identified, almost nothing is known about the factors inducing programmed death of cardiac cells. Oxidative stress induced by oxygen free radicals (ROS) is intimately involved in the development of the phenotype of the failing heart, particularly since cardiomyocytes are characterized by a high amount of oxidative phosphorylation. Notably, the lack of scavenging enzymes leads to severe and lethal dilative cardiomyopathy in knock-out mice. In cell culture models we have been able to characterize the intracellular signaling cascade in cardiomyocytes or vascular smooth muscle cells exposed to oxidative stress ultimately leading to apoptosis.

Furthermore, the role of ROS in p53-induced apoptosis has been investigated. The results show that not Bax, but ROS, are the downstream mediators of p53-induced apoptotic signaling.

It is of great importance to understand how terminally differentiated and post-mitotic cells like cardiomyocytes, can undergo programmed cell death, since it is generally believed that apoptosis is restricted to proliferating cell types due to the fact that it only can occur during a specific and limited phase of the cell cycle. This indicates that there has to be a tight functional interrelationship between the control of cell death, cell growth and the cell cycle in cardiomyocytes. Therefore, we recently investigated the effect of overexpression of E2F-1, which is a key factor in cell cycle control, on the function of cardiomyocytes. Cultured rat cardiomyocytes infected with an adenovirus harboring the E2F-1 cDNA start to initiate the cell cycle machinery, as reflected by an increased expression of S-phase specific genes. However, the vast majority of these cells undergo apoptosis before entering the S-phase. In contrast, cardiomyocytes overexpressing E2F-1 overcome the apoptotic signaling cascade and initiate DNA-synthesis when insulin-like growth factor I (IGF-I) is added to the culture medium. This is of particular interest since, in the heart, IGF-I functions as the exclusive downstream mediator of growth hormone, which is currently used in clinical trials in patients with congestive heart failure.

In order to elucidate the cardiac cell cycle control more systematically, we have developed a myocardial cell-free system where nuclei of terminally differentiated cardiomyocytes are exposed to cellular and/or nuclear extracts of proliferating cells, leading to the reinduction of cardiomyocyte nuclear DNA synthesis.

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Immunology of Cardiovascular Diseases

Gerd Wallukat

Our laboratory is engaged in clinical and basic research mainly focused on immunological processes in cardiovascular diseases. We have observed autoantibodies against adrenergic receptors and AT₁-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 like the corresponding pharmacological agonists. In patients with myocarditis and dilated cardiomyopathy, but also in Chagas' disease, the autoantibodies recognize the β_1 -adrenoceptor and muscarinic M2 receptor as an antigen. In patients with hypertension, the autoantibodies are directed against the α_1 -adrenergic receptor and/or AT₁-receptor.

In recent years, we have investigated in more detail the effects of β_1 -adrenoceptor autoantibodies. These human autoantibodies cross-react with the rat β_1 -adrenoceptor and exhibit their effects *via* the β -adrenoceptor - adenylate cyclase - protein kinase A - cascade. Long-term treatment of cultured rat cardiomyocytes with this antibody leads to a subtype specific reduction of the expression of the β_1 -adrenoceptor on mRNA and protein levels and to upregulation of the inhibitory G-protein Gi α .

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 disappearance of the anti- β_1 -adrenoceptor autoantibodies from the blood.

Based on our autoimmune hypothesis, we have proposed new therapeutic possibilities 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 is observed. This improvement is not only observed immediately after treatment, but for a long period thereafter. In these patients treated with standard therapy plus β -blocking agents, after immunoadsorption, autoantibodies were not detectable and the ejection fraction (EF) increased from 22.3 % before treatment to 37.0 % after a period of 12 months.

The strong correlation observed between the reduction in the amount of circulating autoantibodies to the β_1 -adrenoceptor and the improvement in the function of the heart just described can be interpreted as supporting the hypothesis that the anti- β_1 -adrenoceptor antibodies play a part in the pathophysiology of myocarditis and DCM.

To confirm this hypothesis, we have now developed an immunoadsorption column that selectively removes only the anti- β_1 -adrenoceptor autoantibodies.

Autoantibodies in hypertension

Furthermore, we have 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 α_1 -adrenergic receptor. These autoantibodies and anti-peptide antibodies generated against peptides corresponding to the first or second extracellular loop of the α_1 -adrenergic receptor recognize both these extracellular loops and act like an α -adrenergic agonist.

In a special type of hypertension – preeclampsia – we have observed autoantibodies against the angiotensin II AT₁-receptor. This antibody is detectable after the 20th week of pregnancy and disappears after delivery. The anti-AT₁-receptor antibodies act like the agonist angiotensin II and induce the formation of the AP₁- complex. These functional autoantibodies are found in all preeclamptic women investigated 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 of our research topics is cardiac mast cells. Because the heart of patients with dilated cardiomyopathy contain four times more mast cells and more histamine than controls, we wish to discover 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 have been able to differentiate the mast cells. These differentiated mast cells, mostly located in intimate contact with cardiomyocytes, synthesize the mediators histamine, serotonin and tumor necrosis factor α (TNF α). In heart tissue of DCM patients, we have investigated the degranulation of TNF α and tryptase from mast cells. Both mediators may be involved in the development of fibrosis in the failing heart.

Selected Publications

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Molecular Immunology and Gene Therapy

Thomas Blankenstein

Interleukin 4-deficient mice reconstituted with wild-type bone marrow fail to produce normal immunoglobulin E levels

The ability to reconstitute interleukin (IL)-4^{-/-} mice with bone marrow from IL-4^{+/+} mice was investigated. The absence of the IL-4^{-/-} gene in donor or recipient cells did not impair the reconstitution. All immunoglobulin (Ig) subsets occurred at normal serum levels, except for IgE and to some extent IgG1. IgE production did not recover in the reconstituted mice over a long period. However, these mice were competent for IgE production, because a single intrasplenic injection of IL-4 restored IgE levels, which then remained constant. Wild-type mice reconstituted with wild-type bone marrow maintained IgE serum levels comparable with untreated animals. In wild-type mice reconstituted with IL-4^{-/-} bone marrow, IgE levels decreased gradually and disappeared after 12 weeks. We have made three unrelated, but nonetheless important, conclusions:

(a) (*immunoregulation*) the tightly regulated IL-4 gene is expressed continuously in low amounts (and with apparent absence of antigen stimulation) to maintain the normal threshold of IgE; (b) (*ontogeny of the immune system*) an early unidentified source of IL-4 is postulated which is lost in adult mice; and (c) (*bone marrow transfer/gene therapy*) under certain circumstance, the genotype of the recipient influences the reconstitution.

B cells inhibit induction of T cell-dependent tumor immunity

Cytotoxic T lymphocyte (CTL) mediated tumor immunity against

major histocompatibility antigen (MHC) class I⁺ but class II⁻ tumors often requires help from CD4⁺ T cells. These CD4⁺ T cells are activated by MHC class II⁺ cells that present tumor derived antigens. Considering that different antigen presenting cells (APC), such as B cells, macrophages and dendritic cells, compete for antigen and influence the outcome of an immune response, we have examined tumor immunity in B cell deficient mice and showed that the low immunogenicity of tumors is caused by B cells whose presence in the priming phase results in disabled CD4⁺ T cell help for CTL-mediated tumor immunity. Instead, in the presence of B cells, a non-protective humoral immune response is induced. Our results may explain the enigmatic observation that tumor-reactive antibodies occur frequently in cancer patients.

Direct and indirect T cell priming by dendritic cell vaccines

The mechanisms by which dendritic cell (DC) vaccines prime host T cells *in vivo* has been examined. Mice were immunized with syngeneic bone marrow-derived DC and β-galactosidase (β-gal) was used as a surrogate antigen. DC, either pulsed with peptide, loaded with β-gal antigen or gene-modified, induced β-gal-specific CTL and moderate rejection of an *in vivo* challenge with β-gal expressing tumors. In addition, β-gal-specific CTL lysed the syngeneic DC that were used as vaccines. Using SCID mice reconstituted with F1 lymphocytes, direct priming by gene-modified DC vaccines was demonstrated by the presence of β-gal-specific CTL of the haplotype exclusively expressed by DC, while indirect priming by host APC was shown by the detection of CTL of the haplotype exclusively present on host APC and absent on DC vaccines. DC *in vitro* by lymphokine-activated killer cells, DC vaccines appear to interact with host natural killer cells as well as with antigen-specific T cells. These effector cells, in turn, may lyse DC vaccines, thereby, leading to the release of antigens that can be taken up by host APC.

TH1 associated and cytotoxic T lymphocyte-mediated tumor immunity is impaired in IL-4 deficient mice

Cellular immune responses are induced by CD4⁺ T helper 1 (Th1) cells secreting interleukin (IL)-2 and interferon (IFN)-γ. Tumor immunity is often mediated by CTLs whose activation is supported by Th1 cytokines. Since IL-4 directs Th2 development, and has been shown to inhibit Th1-dominated responses, we have assumed that IL-4-deficient (IL-4^{-/-}) mice would develop vigorous CTL-mediated tumor immunity compared with IL-4-competent (IL-4^{+/+}) mice. Surprisingly, IL-4^{-/-} mice exhibited a severely impaired ability to develop tumor immunity. The lack of tumor immunity in IL-4^{-/-} mice was associated with reduced IFN-γ production, diminished levels of tumor-reactive serum IgG2a, and undetectable CTL activity, indicating a defective Th1 response in the absence of endogenous IL-4. Anti-IL-4 monoclonal antibody blocked tumor immunity in IL-4^{+/+} mice when administered at the time of immunization but not at the time of challenge. Additionally, tumor immunity could be induced in IL-4^{-/-} mice, if IL-4 was provided by gene-modified cells together with immunizing tumor cells. These results demonstrate that tumor immunity requires IL-4 in the priming phase for the generation of effector cells rather than for their maintenance. Together, our results demonstrate a novel, and previously unanticipated, role of IL-4 in the generation of Th1-associated, CTL-mediated tumor immunity.

Retroviral gene transfer

We have constructed retroviral vectors carrying marker genes such as β-galactosidase and green fluorescent protein and have optimized retroviral gene transfer into different cell types. We have analyzed the retrovirus receptor expression on different human tissues and cell lines and shown that the amount of receptor expression does not correlate with the transduction efficiency of three retrovirus vector pseudotypes (A-MuLV, GALV, 10A1) using these receptors for cell entry. We have generated retrovirus vectors carrying 'suicide' genes encoding cytosine deaminase and HSV thymidine kinase and transferred these genes into

different murine and human tumor cells. We have found that the 'suicide' gene/prodrug effect depends on the tumor model and that a double 'suicide' gene approach is superior to single suicide gene activation both *in vitro* and *in vivo*. Successful 'suicide' gene/prodrug treatment requires host immune competence.

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Cellular Immunology

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The newly established group will start operation in spring 2000. One of the major areas of interest is the investigation of mechanisms relevant for the induction or suppression of immune tolerance.

The thymal selection of T cells, in concert with peripheral tolerance mechanisms, generates and maintains a T cell repertoire, which responds to foreign antigens but not to peptides derived from self-proteins. Under certain circumstances, however, these tolerance mechanisms can fail. Autoreactive T cells escape the selection-process and cause the induction of chronic autoimmune diseases, such as multiple sclerosis, diabetes mellitus or rheumatoid arthritis. In most of these autoimmune diseases the typical tissue-specific damage is caused by the activity of autoreactive CD4⁺ T cells (and not by CD8⁺ T cells). In the case of experimental autoimmune encephalomyelitis (EAE) the CD4⁺ T cells are responsible for the removal of the isolating myelin sheath from the axons of the CNS. They inflict the damage either by a direct attack of the myelin sheath or indirectly by activating B cells, which produce myelin-specific antibodies. While in these chronic autoimmune diseases the effect of autoreactive CD4⁺ T cells is extremely harmful, it could be very beneficial if the response could be directed against self-proteins expressed in transformed tissue.

In order to break tolerance it is necessary to increase the sensitivity of the T cells to the autoantigen. One way is to manipulate co-stimulatory signals. For instance, the blockade of CTLA-4, an attenuator of the T cell activation cascade, can significantly lower the threshold for the T cell activation. However, a general

disadvantage of this approach is the complete lack of selectivity. Our efforts, therefore, concentrated on ways to address autoreactive T cells in an antigen-specific manner. In previous studies we showed that multimers containing repeats of a peptide antigen derived from the influenza hemagglutinin protein can trigger an antigen-specific T cell response at almost 1000 fold lower concentrations than the peptide. The enhancement was strictly antigen-specific and appeared to result from the cross-linking of MHC/peptide/TCR complexes. To adapt this approach to a true autoimmune model system multimerized forms of encephalitogenic peptides derived from the myelin basic protein (MBP) or proteolipid protein (PLP) were generated and tested in the EAE system. The trials revealed that the multimerization increased the *in vivo* potency of these epitopes to such an extent that EAE was induced even in strains of mice normally not effected by the monomeric peptides. Furthermore utilizing immunization protocols, which aim at a tolerization rather than the activation of the T cells, relatively small amounts of these multimers were found to be sufficient to suppress the disease (the monomeric peptides did not show any suppressive effect). *In vitro* experiments in the hemagglutinin system indicated that this suppression results from the apoptotic elimination of overstimulated CD4⁺ T cells ('high-zone tolerance'), one of the main mechanisms of peripheral tolerance.

Another tool to enhance the sensitivity of CD4⁺ T cells was found during biochemical studies of two conformational variants of MHC class II/ligand complexes. Binding experiments revealed that the on-rate for the formation of the peptide/MHC complex was significantly increased if certain small molecular compounds were present during the binding reaction. Subsequent studies revealed that these compounds catalyze the ligand exchange in mechanism similar to HLA-DM molecules. In contrast to HLA-DM, however, these compounds facilitated the peptide exchange directly on the surface of antigen presenting cell, increasing the sensitivity of an antigen-specific T cell response by almost 2 logs. This project is still at an early stage and will be continued utilizing combinatorial chemistry. The control of autoimmune reactions is crucial for

the treatment of autoimmune diseases as well as for the development of tumor immunotherapies. To achieve this goal the group will continue to investigate the underlying mechanisms *in vitro*, *in vivo* as well as on the molecular level.

Selected Publications

Röttschke, O., Falk, K., and Strominger, J. L. (1997) Super-activation of an immune response triggered by oligomerized T cell epitopes. *Proc. Natl. Acad. Sci. U S A.* 94, 14642-14647.

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Falk, K., Röttschke, O., Santambrogio, L., Dorf, M. E., Brosnan, C., and Strominger, J. L. (2000) Induction and suppression of an autoimmune disease: Enhanced *in vivo* potency of oligomerized T cell epitopes. *J. Exp. Med.* 191, 1-14.

Structure of the Group

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Molecular and Cell Biology of Hematopoietic Cells

Martin Zenke

The focus of research of this group is the molecular and cell biology of hematopoietic cells. Two hematopoietic cell types are studied in detail: erythroid cells and antigenpresenting dendritic cells (DC). Both cell types are analysed in experimental model systems (mouse and chicken); human erythroid cells and human DC are also being investigated. Additionally, gene transfer methods are being developed to generate gene-modified hematopoietic cells for therapeutic use in medicine.

The thyroid hormone receptor/c-erbA (TR/c-erbA) acts as a binary switch in red blood cell development

P. Bartunek, G. Blendinger, M. F. Heikenwälder, and S. M. Kurz

The c-erbA protooncogene product represents a high affinity receptor for thyroid hormone (thyroid hormone receptor, TR). Our previous work has established that TR/c-erbA induces red cell-specific gene expression and effectively accelerates erythroid cell differentiation when activated by ligand (Zenke et al., Cell 61, 1035). We have now found that additionally unliganded TR/c-erbA affects erythroid cell development; it supports sustained growth of erythroid progenitor cells *in vitro* by blocking differentiation (Bartunek and Zenke, 1998) and, therefore, exhibits an activity very similar to its oncogenic version v-erbA (Zenke et al., Cell 52, 107). Thus, TR/c-erbA acts as a binary switch in determining the fate of the erythroid cell: unliganded TR/c-erbA supports growth while ligand-activated TR/c-erbA induces differentiation. Our activities are now being directed towards the identification of TR/c-erbA target genes (in collaboration with P. Pajér and M. Dvorák, IMG, Prague, Czech Republic). To this end, several potential erbA target genes have been isolated and are currently being analysed.

Determining the gene expression repertoire of red blood cells.

N.P. Koritschoner, B. Anzinger, G. Blendinger, S. Knespel, B. Lemke, and P. Bartunek

In initial studies, the tyrosine kinase gene expression profiles in erythroid progenitors and differentiated cells were determined by employing gene family PCR and targeting the highly conserved tyrosine kinase domain. Several receptor and non-receptor tyrosine kinases have been identified that undergo specific changes in expression when cells differentiate (Koritschoner et al., 1999). These experiments led to the identification of fibroblast growth factor receptor-4 (FGFR-4) as a new ligand-dependent regulator of erythropoiesis.

To extend these studies, we have now established an *in vitro* differentiation

system for human red blood cells (Panzenböck et al., 1998; in collaboration with M. Mapara, Charité, Robert-Rössle-Klinik, Berlin). Erythroid precursor cells from cord blood, CD34⁺ stem cells or bone marrow are amplified *in vitro* in the presence of stem cell factor (SCF), erythropoietin (Epo), dexamethasone and estrogen, and differentiated by Epo and insulin treatment. Specific changes in gene expression during differentiation are monitored. This experimental system provides the basis for determining the entire gene expression repertoire of human red blood cells by DNA chip technology. These studies are currently being performed.

Gene expression in antigen-presenting dendritic cells (DC)

J. Madruga, S.M. Kurz, S.S. Diebold, N.P. Koritschoner, S. Knespel, M. Meyer, and X.-S. Ju

Dendritic cells (DC) are professional antigen-presenting cells that are unique in that they can initiate primary immune responses. However, so far, many of the functional and molecular properties of DC are 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). Using this system, molecular mechanisms of DC motility were investigated (Madruga et al., 1999). Several components of focal adhesion complexes are expressed in v-relER DC that are, however, not organized in classical focal adhesion plaques, but rather exhibit a polarized expression pattern and colocalize with actin. Additionally, the expression and function of receptor tyrosine kinases in DC are being assessed.

To gain further insight into the underlying mechanisms that determine DC differentiation, an *in vitro* system for differentiation of human DC from hematopoietic stem/progenitor cells has been developed. Cells are grown with a stem cell factor cytokine cocktail that maintains the progenitor phenotype, and 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 further mature by TNF α or CD40L. Additionally, upon differentiation induction DC cease proliferation and effectively undergo cell cycle arrest. The expression of various cell cycle regulators and transcription factors in differentiating DC is now being investigated.

Gene transfer into antigen-presenting dendritic cells (DC)

Diebold, S. S., Esslinger, C. and Gust, T. C.

Given their unique properties in antigen-specific T cell activation, DC represent a particularly attractive cell type for use in the immunotherapy of diseases such as cancer. 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. To develop DC for medical therapy, gene-modified DC have been 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).

DC abundantly express mannose and adenovirus receptors. Accordingly, mannose polyethylenimine (ManPEI) conjugates were synthesized consisting of the receptor binding moiety mannose and the polycation PEI that binds and condenses DNA and, following uptake into cells, facilitates exit from the endosomal compartment. Additionally, Ad/PEI/DNA transfection complexes have also been generated that contain plasmid DNA bound to the outside of adenovirus particles by PEI, with adenovirus particles serving as the ligand for receptor-specific uptake. Both ManPEI/DNA and Ad/PEI/DNA transfer complexes are effective in delivering DNA into human and mouse DC and eliciting specific T cell responses (Diebold et al., 1999, 1999a; 1999b). The activity of gene-modified mouse DC is being studied both *in vivo* and *in vitro*. The Ad/PEI and ManPEI gene delivery systems are particularly versatile and should be useful for the generation of gene-modified DC to be employed in

medical therapy and to study DC biology and function.

Selected Publications

Bartunek, P., and Zenke, M. (1998) Retinoid X receptor and c-erbA/thyroid hormone receptor regulate erythroid cell growth and differentiation. *Mol. Endo.* 12, 1269-1279.

Panzenböck, B., Bartunek, P., Mapara, M., and Zenke, M. (1998) Growth and differentiation of human stem cell factor/erythropoietin-dependent erythroid progenitor cells *in vitro*. *Blood* 92, 3658-3668.

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Phospholipids

Dietrich Arndt

Cytotoxic effects of antitumor agents induced at the plasma membrane level

We are investigating the anticancer properties of special phospholipids. These compounds, ether lipids and alkylphospholipids (APLs), represent a new class of non-DNA-interactive compounds for cancer therapy. They act as growth factor antagonists, growth factor receptor blockers, and interfere with mitogenic signal transduction, modulate phospholipid turnover, induce differentiation and apoptosis and activate macrophages to a tumoricidal state.

The aim of our research is to investigate the correlation between antineoplastic activity and the supramolecular structure of phospholipids with antitumor properties. Thus, we are concentrating mainly on the investigation, characterization and use of liposomes prepared from phospholipids with inherent antineoplastic activity

Antineoplastic activity of alkylphospholipid liposomes in human breast carcinomas

We have developed sterically stabilized APL liposomes which avoid uptake by the reticuloendothelial system and can be targeted passively to tumor tissue by increased microvascular permeability in the tumor area. The bilayer of such sterically stabilized liposomes consists of hexadecylphosphocholine, cholesterol and polyethylene glycol-linked phosphoethanol-amine. The reduced uptake of sterically stabilized APL liposomes correlates *in vitro* (J774 cells) with an increased thickness of the fixed aqueous layer around these liposomes and supports

the hypothesis that the thickness of this aqueous layer is an important factor responsible for preventing opsonization, thereby resulting in reduced macrophage uptake. The pharmacokinetics of free and different liposomal APLs is in agreement with these assumptions; the serum levels of APL obtained with sterically stabilized liposomes are consistently higher than with conventional vesicles and free APL. In xenografted MaTu carcinoma, the differences in APL content between the different groups are unexpectedly low and do not reflect the high therapeutic activity of sterically stabilized APL liposomes. Detailed analysis shows that the liposomal drug displays modified pharmacokinetics which may also involve lymphatic absorption of the liposomal APL.

The physical properties and pharmacological activity of liposomes made from a new, highly active alkylphospholipid (OPP) have been optimized with special reference to the composition of the vesicles. The strongest antitumor effect on xenotransplanted human breast cancer MT-3 on nude mice was obtained with sterically stabilized OPP liposomes with a low cholesterol content. The beneficial therapeutic effect of these vesicles was accompanied by better tolerance and a significant inhibition of hemolysis, compared with micellar OPP.

Immunoliposomes from alkylphospholipids

For active targeting, we have investigated the preparation of immunoliposomes using hexadecylphosphocholine and the monoclonal antibody fragment 4D5, specific against the p185^{HER2} protein, a growth factor receptor-tyrosine kinase. Conjugation was achieved by coupling the protein *via* a thioether linkage to the liposomal surface. To investigate the cellular uptake and endocytosis by tumor cells, a pH-sensitive fluorescence marker was encapsulated into the liposomes. Experiments *in vitro* demonstrated a difference in binding of liposomes, with and without antibody, to cells with different receptor expressions. For active targeting of the endothelium of the tumor neovasculature, we are investigating the preparation of immunoliposomes using APL and peptides that

specifically target distinct blood vessels. Each of these peptides binds to different receptors that are selectively expressed on the vasculature of the target tissue. The tumor-binding peptides, e.g. peptides containing an integrin-binding Arg-Gly-Asp motif or the Asn-Gly-Arg motif, bind to receptors that are upregulated in tumor angiogenic vasculature.

Selected Publications

Zeisig, R., Arndt, D., Stahn, R., and Fichtner, I. (1998) Physical properties and pharmacological activity *in vitro* and *in vivo* of optimised liposomes prepared from a new cancerostatic alkylphospholipid. *Biochim. Biophys. Acta* 1414, 238-248.

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Patent Application

DE 198 55 953.4. Mittel zur Tumorthherapie. Erfinder: Zeisig, R., Fichtner, I., and Arndt, D.
DE 198 55 952.6 Mittel zum nichtviralen Transfer von DNS in eukaryotische Zellen. Erfinder: Zeisig, R., and Walther, W.

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RNA Chemistry

Eckart Matthes

Two target inhibitors of human telomerase (hTERT)

Telomerase is a unique ribonucleoprotein polymerase using its RNA component as a template for the synthesis of multiples of telomeric repeats onto the end of replicating chromosomes. The extension mechanism of telomerase compensates for the loss of telomeric DNA associated with each round of DNA replication. However, most somatic cells lack telomerase and so the telomere length reduction is thought to limit their proliferative capacity and to lead to cellular senescence.

On the other hand, an activation of telomerase seems to be required for the sustained growth potential of malignant tumor cells, stem cells of renewable tissues and germ cells. Telomerase activity was detected in 85-95 % of advanced malignant tumors.

These findings make telomerase an attractive target for anti-neoplastic drugs. One promising target might be the RNA molecule of telomerase which is an integral part of the enzyme. Indeed, it has been shown that oligonucleotides (ODNs) covering the template site of RNA are able to control the growth of tumor cells.

Telomeric DNA has been suggested to bind not only to the template RNA but seems also to be attached via its 5'-end to a telomerase protein site called the primer binding site. We have found that this protein site is an appropriate target for inhibition of telomerase. This possibility emerged from our investigations of differently modified ODNs. Of these, phosphorothioate-modified ODNs (PS-ODNs) were found to be the most

efficient inhibitors compared with other oligomers including peptide nucleic acids (PNA). We found that telomerase protein, rather than its RNA, is the target of PS-ODNs, a property which has proved to be more length- than sequence-dependent. This mode of action seems to lead to a higher efficiency of PS-ODNs compared with antisense oligomers targeting telomerase RNA. The concentration required for 50 % inhibition of telomerase in HL-60 cell-lysates was found to be in the nanomolar range.

To increase the selectivity of PS-ODN, we designed chimeric ODNs (cODNs) which are extended at the 3'-end by an oligomer hybridizing effectively with the subsequent template region of RNA. Furthermore, such cODNs address two different targets of telomerase and might be more efficient. Most of these cODNs have been synthesised by our group and optimized by length-variations (10-20mers) of the PS-part and by length- (5 and 11 mers) and structural-modifications of the antisense part (e.g. 2'-methoxy, 2'-methoxyethoxy, phosphoramidate). PS-PNA chimeric oligomers were provided by Dr. E. Uhlmann, Hoechst Marion Roussel. Our results show that cODN are more effective than pure PS-ODN and inhibit human telomerase in the subnanomolar range. Complexed with lipofectin, cODNs can be taken up by U87 glioblastoma cells and effectively inhibit telomerase. The concentration required for 50 % inhibition of telomerase inside U87 cells is 0.05 - 0.3 μ M.

Therefore, we consider our cODNs to be useful candidates for *in vivo* applications to investigate the consequences of permanent inhibition of telomerase on the growth of human tumors in nude mice.

Selected Publications

von Janta-Lipinski, M., Costisella, B., Ochs, H., Hübscher, U., Hafkemeyer, P., and Matthes, E. (1998) Newly synthesized L-enantiomers of 3'-fluoro-modified β -2'-deoxynucleoside 5'-triphosphates inhibit hepatitis B DNA polymerases but not the five cellular DNA polymerases α , β , γ , δ , and ϵ nor HIV-1 reverse transcriptase. *J. Med. Chem.* 41, 2040-2046.

Theil, F., Ballschuh, S., Flatau, S., von Janta-Lipinski, M., and Matthes, E. (1998) Chemoenzymatic synthesis of a new type of enantiomerically pure carbocyclic nucleoside analogues with strong inhibitory effects on terminal deoxynucleotidyl transferase. *Bioorg. & Med Chem.* 41, 701-706.

Matthes, E., and Lehmann, Ch. (1999) Telomerase protein rather than its RNA is the target of phosphorothioate-modified oligonucleotides. *Nucleic Acids Res.* 27, 1152-1158.

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Patent Application

Matthes, E., and von Janta-Lipinski, M. Chimeric oligonucleotides and the use of thereof PCT-Application: PCT/DE98/01216; 4th May, 1998.

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Regulation and Deregulation of Cell Proliferation and Gene Therapy

Research Group of the Humboldt University of Berlin at the MDC

Regulation of cell proliferation

S. Boeckh, S. Schlisio

The cell cycle research in our group has so far focused on the role of the retinoblastoma protein (pRb) in the G1-checkpoint, the so-called restriction point, controlling the switch between alternative cellular fates. Referring to its role as a transcriptional regulator, various extracellular matrix genes, thrombospondin and cyclin D1, among others, have been identified as pRb-responsive genes. Dissecting the cyclin D1 promoter in more detail, we provided evidence for a direct link between NF- κ B activity and cell cycle regulation by demonstrating transcriptional activation of cyclin D1 by NF- κ B (work by Michael Hinz).

In order to also evaluate the integration of antimitogenic pathways in the pRb pathway, we have recently started to investigate the relationship between TGF- β signalling and cell cycle control. TGF- β 's are potent growth suppressors in many different normal cell types whereas, in contrast, many cancers are resistant to TGF- β . In many cancer cell lines, especially those of the pancreas and colon, impairment of the TGF- β pathway, as manifested by genetic alterations of TGF- β 's, their receptors, or downstream targets, in addition to a deregulated pRB pathway, has been observed. In order to understand the acquisition of a malignant phenotype in pancreatic carcinoma we are aiming to identify alterations of gene expression induced in pancreatic and colon cancer cells by TGF- β , especially at early time points. We are using "high density cDNA filters", provided by the Resource Center of the German Human Genome Project. So far, our screening experiments have identified more than 25 distinct cDNA clones as potential TGF- β target genes. However, many of them are regulated in response to the TGF- β induced cell cycle arrest, as confirmed by Northern Blot and FACS analysis. To select the 'real' TGF- β targets we are now screening cells with a reconstituted TGF- β pathway. Judging from our results so far, this approach is completely reproducible in our hands and may help us understand the effect of a signalling cascade on the expression profile of cancer cells.

Gene therapy of familial hypercholesterolemia

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

A second research project of our group is the development of gene transfer systems for the correction of monogenetic diseases affecting normal liver function. In animal models for *Familial Hypercholesterolemia* (FH), a disease caused by an inborn malfunction of the *low density lipoprotein receptor* (LDL-R), the efficiency of viral vector systems and vector-related side-effects are under investigation. The application of recombinant adenoviruses, carrying a functional LDL-R, leads to normalisation of serum cholesterol levels in Watanabe rabbits (animal model for FH), but the therapeutic effect is only short-term (10-14 days) and accompanied by acute hematological changes (thrombocytopenia, anemia, erythroblastosis). The hematological side-effects are a result of a rapid systemic distribution of viral vectors, which cannot be controlled by local vector administered *via* the portal vein. Beside acute hematological changes, adenoviral vectors induce adverse immunological reactions in mammals which interfere with transgene expression and could induce inflammatory changes in the liver and other organs. Pharmacological immunosuppression leads to prolongation of the therapeutic effects but is not a preferred solution as far as the induction of lymphoproliferative disorders and the permanently suppressed immune state are concerned. Less immunogenic vectors providing long-term gene expression are required. We are currently focussing on the use of lentiviral vectors for liver gene transfer. To overcome the current titer problems in lentiviral vector technology, we are developing chimeric adenovirus-lentivirus vectors for *in vivo* release of recombinant lentiviruses.

Transient immunosuppression with 15-deoxyspergualin prolongs reporter gene expression and reduces humoral immune response after adenoviral gene transfer. Cichon G., Strauss M. *Gene Ther.* 1998 Jan; 5(1): 85-90

Intravenous administration of recombinant adenoviruses causes thrombocytopenia, anemia and erythroblastosis in rabbits. Cichon G., Schmidt H.H., Behdidjeb T., Loser P., Ziemer S., Haas R., Grewe N., Schnieders F., Heeren F., Manns M.P., Schlag P.M., Strauss M. *Journal of Gene Medicine* Sept-Oct 1999; 1(5): 360-371

Gene therapy of malignant tumors

Karsten Brand, Sefer Eleskurtaç, Martina Geheeb, Christina Montag, Ansiah Shakeri-Garakani

This project group deals mainly with the development of new methods for the virus-based gene therapy of tumors, especially colorectal liver metastases. We are pursuing three approaches:

1. *Gene therapeutic chemotherapy by intratumoral gene transfer of the Herpes Simplex Virus thymidine kinase gene which toxifies intravenously administered Ganciclovir.*

In previous studies, we have described the toxicity 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 examining diverse ameliorated tumor tissue specific promoters in the context of this new vector generation with the aim of obtaining a clinically relevant therapy with high efficacy but low toxicity.

2. *The transfer of cell cycle-inhibitory and apoptosis-inducing genes.*

In our previous work, we were able to show the therapeutic relevance of the simultaneous expression of more than one gene of these classes of proteins. We are currently examining 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. *The 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-associated proteases leads to a dramatically reduced 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 with the aim of combining this very efficient approach with the lowest possible toxicity.

Selected Publications

Brand, K., Löser, P., Arnold, W., Bartels, T., and Strauss, M. (1998) Tumor cell-specific transgene expression prevents liver toxicity of the adeno-HSV-tk/GCV approach. *Gene Therapy* 5, 1363-1371.

Brand, K., Klocke R., Poßling, A., Paul, D., and Strauss, M. (1999) Induction of G2/M arrest and apoptosis by replication deficient adenovirus. *Gene Therapy* 6, 1054-1063.

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Evolution, Regulation and Genetic Applications of Transposable Elements in Vertebrates

Zoltán Ivics

Transposable elements are mobile segments of DNA that are ubiquitous in most living organisms. These elements can be significantly amplified in genomes, which may have a profound impact on genome organization and are, therefore, thought to play a role in speciation. In contrast to viruses, transposons are permanent inhabitants of genomes. Thus, the relationship between transposon and the host genome is delicately controlled. The types, numbers, conservation and genetic locations of transposable elements provide important clues about the evolution of transpositional mechanisms and their regulation, and the effects of transposons on the expression of host genes. In addition, transposable elements can be harnessed as useful experimental tools for the characterization and genetic manipulation of genomes.

Accordingly, we have embarked on an intensive search to identify and characterize transposable elements in zebrafish (*Danio rerio*), a powerful model system for vertebrate embryogenesis. As a result of our ongoing efforts, approximately 20 % of the complex genome of the zebrafish is now relatively well characterized.

DANA is a composite, tRNA-derived retroposon, which is amplified through an RNA intermediate. Some of the sequence modules that make up the DANA element are apparently capable of forming new groups of mobile, composite transposons. One of these sequences, called MER-6, is an abundant repeat found in the human genome. Both DANA and MER-6 elements contain polymorphic microsatellite CA repeats, raising the possibility that these elements are sources of genomic instability in vertebrate genomes.

Angel is an abundant miniature inverted-repeat transposable element (MITE), dispersed in the zebrafish genome. *Angel* elements are palindromic sequences with the potential to form stem-loop structures *in vitro*. Despite considerable sequence divergence, the inverted repeat structures of these elements have been maintained, implying functional importance. We have proposed a model in which MITEs take advantage of a basic cellular mechanism, DNA replication, for their amplification, which is dependent on the characteristic inverted repeat structures of these elements.

Both DANA and *Angel* elements are particularly suitable as genetic markers because they have high copy-numbers and random distribution in the genome, and segregate in a Mendelian fashion. There are extensive DNA polymorphisms between zebrafish populations and strains detected by PCR amplification using primers specific to DANA and *Angel*. Database searches indicate a high association of *Angel* elements with zebrafish EST's; thus, these elements are excellent markers for genetic mapping.

Tc1/*mariner*-like transposable elements spread through a DNA intermediate, and this process is catalyzed by the element-encoded transposase. Members of this transposon family have been found in several vertebrate genomes; however, all of the transposon copies isolated to date are clearly relics of once active transposons that, after successfully colonizing genomes, have become inactivated by mutations. This inactive state of these elements greatly hinders investigations into the mechanisms, regulation and evolution of DNA transposition in vertebrate species.

Based on a comparative phylogenetic approach, we have reconstructed an active Tc1-like transposon from bits and pieces of inactive elements found in the genomes of teleost fish, and named this transposon *Sleeping Beauty* (SB). SB mediates efficient and precise cut-and-paste transposition in cells of a variety of vertebrate species, including humans. SB is the first active member of the Tc1 family of transposons in vertebrates, and could be identical or equivalent to an ancient element that dispersed in teleost genomes, in part by horizontal transmission between species.

We are concentrating our efforts on the following main research areas.

- 1) Developing high copy number and polymorphic elements (especially DANA and *Angel*) as molecular markers, and establishing a repetitive element database for the zebrafish to facilitate mapping, identification and sequence analysis of genes.
- 2) Transposons have learned how to coexist peacefully for millions of years with their host cells by minimizing the mutational damage they inflict on the host genome. Down-regulation of transposition can be achieved by factors and mechanisms provided by the host cell or by self-limiting regulatory features intrinsic to the transposon itself. We are investigating how transposition is regulated in vertebrates, and the molecular interactions that allow this peaceful relationship to be maintained during evolution.
- 3) *Sleeping Beauty* 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 and develop SB as a molecular tool for human gene therapy, so that it will become a useful vector for inserting therapeutic genes into human chromosomes.
- 4) Exploiting transposons to determine the identity, function and biological relevance of genes that are associated with vertebrate embryonic development and human disease, by developing insertional mutagenesis screens in model organisms such as fish, frogs, and mice.

Selected Publications

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Plasterk, R.H., Izsvák, Zs., and Ivics, Z. (1999) Resident Aliens: The Tc1/*mariner* superfamily of transposable elements. Trends Genet. 15, 326-332.

Patent Application

DNA-based transposon system for the introduction of nucleic acid into DNA of a cell Tc1-based transposon vectors

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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 – the American government has indeed declared the nineties the “Decade of the Brain” – considerable progress remains to be made in the elucidation of those molecular events that are responsible for brain disorders.

It is due to the immense complexity of the brain that progress in understanding the molecular processes that govern its function is slow. However, two different approaches have been developed to combat this problem: positron emission tomography (PET) and nuclear magnetic resonance imaging (NMRI) enable neuroscientists to visualize and map active centers of the brain in relation to specific functions, and molecular and cell biological tools are being 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 to design new treatments for the specific degenerative mechanisms which lie at the root of many brain diseases.

The research groups of the MDC's neuroscience program are using molecular and cellular approaches. Cellular Neurosciences, headed by Helmut Kettenmann, is investigating the role of glial cells in health and disease, while Developmental Neurobiology, under the direction of Fritz G. Rathjen, is analyzing the molecular aspects of axonal growth during the development of the central nervous system. Gary R. Lewin and his coworkers are focussing on identifying novel genes responsible for mechano-transduction and their regulation by neurotrophins, while Frank W. Pfrieger is interested in factors which control synaptogenesis.

The central theme of the MDC is to link basic and clinical research and we have, therefore, established cooperations 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. These interactions are being fostered since we are part of the Collaborative Research Center (Sonderforschungsbereich) at the Charité established to study the role of non-neuronal cells in the pathogenesis of CNS diseases.

Helmut Kettenmann

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 electrical activity in the brain. As a consequence, previous neuroscience research 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 have brought these cells into the arena of neuroscience. 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 and ensure rapid signal conduction in the white matter. The role of astrocytes is less well defined; they provide guiding structures during development and are important elements for controlling the composition of the extracellular space, mediating signals between brain endothelium and neuronal membrane. Microglial cells in the brain are immunocompetent and their functional role is best defined as the first elements to respond to pathologic events. While in recent years the group has studied aspects of all three types of glial cells, the present research program is 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 respective group grant from the German Research Council (Sonderforschungsbereich 507, 515, Schwerpunktprogramm on Microglia).

1. Do astrocytes express receptors for neurotransmitters and neuromodulators?

In recent years, we have learned that astrocytes in cell culture have the capacity to express almost all receptors known to mediate synaptic transmission. When we analyzed the receptor repertoire in more intact systems, i.e. in freshly isolated brain slices, we observed that defined types of astrocytes express only a restricted pattern of neurotransmitter receptors. One of our best studied examples is the Bergmann glial cell in the cerebellum, a morphologically specialized astrocyte. We have found that these cells express a receptor repertoire similar to the corresponding neurons of that brain region. To facilitate studies in other areas of the central nervous system which do not contain morphologically distinct astrocytes, we have generated a transgenic mouse in which all astrocytes express a green fluorescent protein. Using this approach, we have recently shown that cortical astrocytes express the NMDA-type glutamate receptor, a receptor which has not been found in cultured astrocytes.

2. Do glial cells perceive neuronal activity?

To test whether glial cells have the capability to sense neuronal activity, we have used a cerebellar slice preparation and confocal microscopy, combined with ion concentration imaging, to test for such neuron-glia interactions. We have found that stimulation of parallel fibres, the axons of the granule cells, triggers calcium elevation in Bergmann glial cells. These increases are confined to morphological units which are 5-10 micrometers in length. These units, termed 'microdomains', can even exhibit spontaneous intrinsic calcium activity. We believe that these units could be involved in the feedback of information on a defined population of synapses, i.e. those which are enclosed by a given microdomain.

3. What are the controll mechanisms of microglial activation?

Microglial cells are the major immunocompetent cells in the brain. We have studied the signals which lead to the activation of microglia and have also analyzed the cellular and molecular consequences of this activation. We have found that different stimuli, e.g. confrontation with Gram-positive or Gram-negative bacteria, can lead to different activation patterns in these cells. We have recently developed an *in situ* model which allows us to study the physiological responses of resting and activated microglia. This has enabled us to characterize the functional receptors and the physiological phenotype of these cells. Using this approach we have recently reported that resting microglia expresses a physiological phenotype which is distinct from all other CNS cell types and also shows differences with regard to macrophages.

4. 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 migrate. The cells are analyzed in living brain slices from surgically obtained human material. One of the new interesting aspects of this line of research is the finding that cells in oligodendrogliomas do not exhibit the physiological properties of oligodendrocytes, but have properties more in common with neurons – they are able to generate action potentials. This similarity extends to the finding that the main excitatory neurotransmitter of the central nervous system, glutamate, triggers electrical excitability in the tumor cells. Recently, we have focused on the expression of GABA receptors by glioma cells since we have found a very strong correlation between the expression pattern and 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 now have evidence that the brain environment triggers the induction of this receptor and we will test whether interference with receptor activity influences the behavior of the tumor cells.

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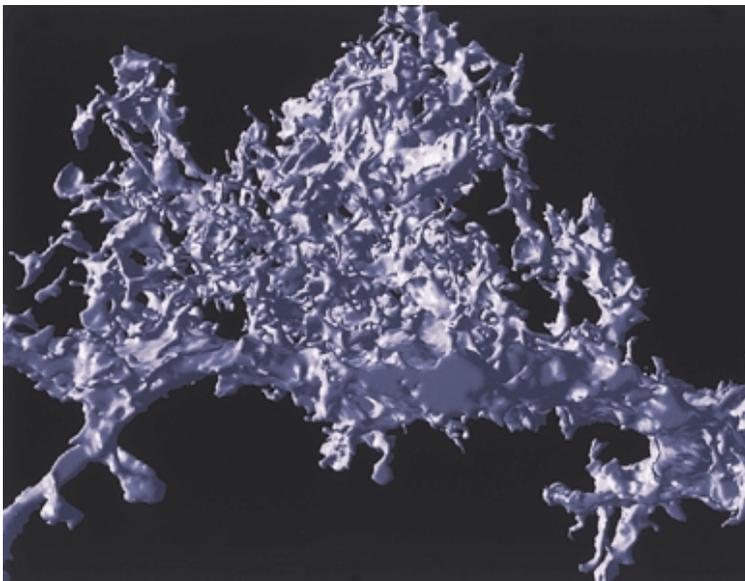


Figure 38: A small section of a Bergmann glial process has been reconstructed based on EM data. Within these structures the neuronal elements are embedded and constitute the site of neuron-glia interaction.

Growth Factor and Regeneration

Gary R. Lewin

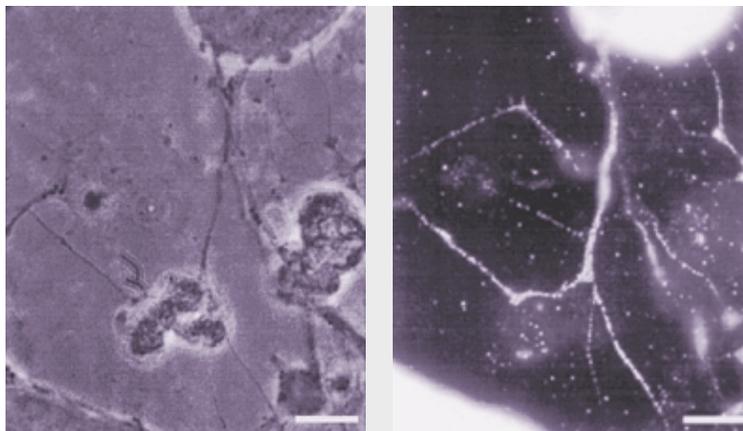
We are using sensory neurons of the dorsal root ganglia as a model system to study neuronal specification. Sensory neurons subserve sensations such as touch and pain and we have shown that different members of the neurotrophin family interact with functionally-distinct sensory neurons to regulate the survival and specific functional properties of sensory neurons. We have also established that brain-derived neurotrophic factor (BDNF) regulates the mechanotransduction properties of slowly-adapting sensory neurons, but not their survival (Carroll et al. 1999).

Molecular basis of mechanotransduction

Our finding that neurotrophins regulate mechanotransduction has led us to concentrate on elucidating the molecular mechanisms underlying mechanotransduction in mammals. Based on a molecular model of mechanotransduction in *C.elegans*, we want to establish whether mammalian homologues of genes essential for touch sensitivity in *C.elegans* (the 'Mec' genes) also function as part of a mechanotransduction complex in mammals. In my lab, we have carried out *in situ* hybridization, Northern blotting and immunocytochemical studies that have established that some species homologues are appropriately expressed in dorsal root ganglion neurons (DRG) (Mannsfeldt et al., 1999). Two mammalian homologues of MEC proteins are stomatin, an integral membrane protein, and mdeg, a sodium channel (MEC-2, and MEC-4 respectively in *C.elegans*). To test whether such genes are functionally involved in mechanotransduction, we are presently making transgenic mice that overexpress these putative mechanotransduction genes in sensory neurons. In addition to these functional studies, we have recently isolated two novel cDNAs encoding new members of the stomatin family and have found that both are highly expressed by mammalian sensory neurons. We also plan to establish whether these novel genes have a functional role in mechanotransduction.

Development of physiologically distinct sensory neurons

Using an *in vitro* electrophysiological preparation, where one can record from identified sensory neurons innervating skin, it is possible to quantify and characterize physiologically distinct sensory neurons. We have taken advantage of this preparation to ask whether different molecules are involved in specifying the numbers of these different receptor types or their properties during development. By taking mice with targeted deletion or replacement of neurotrophins or their receptors, we have been able to show that individual receptor types require individual neurotrophins during their development. In our most recent study carried out in collaboration with Dr. Rüdiger Klein from the EMBL (Heidelberg), we have shown that neurotrophin-4 exclusively supports the survival of one skin receptor type, the D-hair receptor, by activating signalling pathways downstream of the shc binding site on the trk B receptor (Minichiello et al. 1998). In addition to these studies, using patch clamp techniques, we have also recently shown that nociceptive neurons (pain sensing) with different neurotrophic requirements are functionally distinct (Stucky and Lewin, 1999). These studies may be important as we were also able to show that NGF directly regulates the noxious heat sensitivity of some neurons, a mechanism that might be responsible for injury-induced hyperalgesia in humans. Interestingly, the ability of these NGF- and GDNF-dependent populations to sprout is also dramatically different (Belyantseva and Lewin, 1999).



Phase contrast

Mdeg immunofluorescence

Figure 39: Photomicrograph of a cultured adult sensory neuron. The cell has been stained with an antibody directed against the ectodomain of the mdeg channel, a putative mechanotransducing channel. Note that this channel is localized in microdomains on axonal membranes.

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Mannsfeldt A.G., Carroll P., Stucky C.L., and Lewin G.R. (1999) Stomatin, a MEC-2 like protein, is expressed by mammalian sensory neurons. *Mol. Cell. Neurosci.* 13, 391-404.

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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 dynamic exchange of electrical signals between neurons. Our group focuses on the identification of the signals and mechanisms that control the formation and stabilisation of synapses in the mammalian central nervous system (CNS).

Identification of signals controlling synapse formation and maturation in the mammalian CNS

Up to now, the signals that control the formation of synaptic connections between CNS neurons are largely unknown. Recently we presented evidence that macroglial cells secrete a activity that specifically promotes the formation of efficient synapses in cultured CNS neurons. We now aim to identify these signals and characterize their mode of action using primary cultures of purified neurons and a wide range of techniques including electrophysiology, microfluorometry as well as biochemical and molecular biological methods. During the last year, we have been able to take the first steps towards the biochemical purification of these factors and have shown that the synapse-promoting activity is carried by soluble glial proteins which may interact with components of the extracellular matrix. In order to learn more about the neuronal signaling pathways that mediate the glial effects on synapse formation, we have established microcultures of purified CNS neurons, where neurons are cultured on small drops of substrate that confine neuronal outgrowth to a small circular area and, thus, force neurons to make synapses onto themselves (see figure). Using these cultures, we can now study the number, localization and efficacy of synapses in individual neurons and under different culture conditions. Furthermore, we have established purification procedures for different types of CNS neurons and can now ask whether glial factors control synapse development throughout the CNS, or whether different types of neurons have different signaling requirements to form efficient synapses. In the long-term, we would also like to explore the potential of “synaptogenic” factors to repair synaptic connections that have been lost in the wake of neurodegenerative diseases or brain injury.

Mechanisms controlling the life and death of synapses

The selective stabilization and elimination of synaptic contacts are important processes controlling the development, plasticity and functional integrity of the CNS. The uncontrolled loss of synapses is largely responsible for the devastating deficits in brain function caused by neurodegenerative or injury-induced lesions. Despite the importance of synaptic stability, we know very little about the signals controlling the life-time of synapses and the mechanisms leading to their elimination during development and in the adult. We are currently addressing these questions using two testable hypotheses: namely, a) that synapses have an intrinsic ‘expiration date’ that is modified by external and internal signals and b) that synapses are eliminated by apoptotic processes which, under pathologic conditions, may ultimately lead to cell death. We are testing these hypotheses by measuring the life-time of synapses in suitable experimental model systems and determining whether apoptotic signals play a role during synapse elimination. The identification of signals and mechanisms that control the synapse stability and initiate their elimination may help us identify new targets to treat the pathologic loss of synapses.

Automatization of cell purification

Primary cultures of highly purified neurons and glial cells provide an ideal model to study differentiation processes since they permit strictly defined cultures conditions. A major drawback of these models is the rather laborious procedures of cell purification. In collaboration with partners in the biotechnology industry, we aim to develop an integrated system for the automated purification of brain cells. As a first step, we have designed a platform that performs the different steps from tissue dissociation to cell selection. Next, we aim to develop a system prototype that allows functionality tests and design improvements to be carried out.

Selected Publications

Pfriege, F.W., and Barres, B.A.
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(1997) Synaptic efficacy enhanced by glial cells *in vitro*. *Science* 277, 1684-1687.

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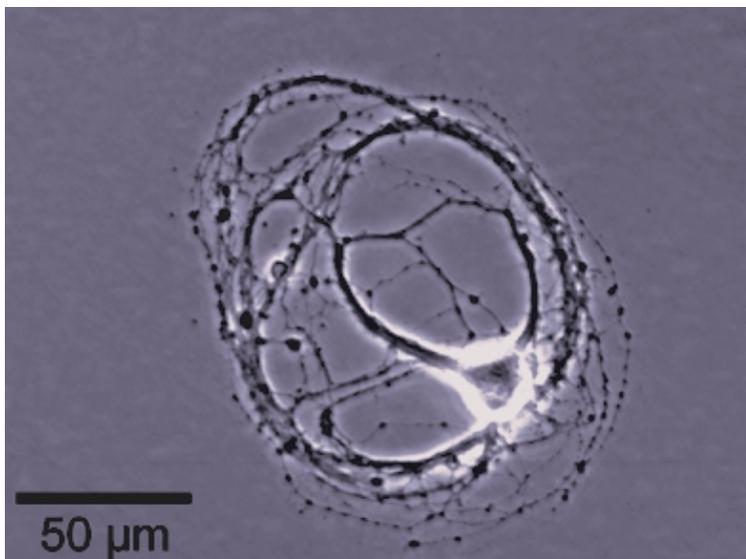
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Figure 40: Figure depicts a retinal ganglion cell, purified from postnatal rats and cultured on a substrate microisland in defined medium for 14 days.



Developmental Neurobiology

Fritz G. Rathjen

Molecular analysis of axonal growth and pathfinding during embryonic development of the nervous system

An interesting and long-standing question is how axons are guided to their target region to establish synaptic connections. During embryonic development and axonal regeneration after injury, neurons respond to an array of molecular signals that are present in the microenvironment of extending axons. These signals activate axonal cell surface receptors and elicit specific growth cone responses. Several classes of proteins have been shown to be implicated in these processes including neural members of the immunoglobulin superfamily (IgSF), semaphorins, netrins, ephrins and their receptors, as well as tenascins and the EGF family of growth and differentiation factors. The most diversified class of proteins that is implicated in contact-dependent regulation of neurite outgrowth and axon guidance are the neural members of the IgSF which can be categorized into several structural subclasses including the L1-, F11- and IgLON-subgroups. Functional *in vitro* studies have been supported by intriguing *in vivo* observations in mice and humans indicating that this class of proteins is important for the correct wiring of the nervous system. Currently, our research is focussing on the *in vitro* and *in vivo* function of members of the IgSF and tenascins, as well on a member of the EGF family of differentiation factors using different model systems.

Neurofascin exerts its function through interactions with multiple heterophilic ligands

The L1 subgroup of the IgSF in vertebrates consists of four members: L1 itself, neurofascin, NrCAM and CHL1. They are transmembrane proteins that have been localized to growth cones and processes of postmitotic neurons where they mediate cell adhesion, neurite outgrowth and axon bundling. Currently we are focussing on two members of this subgroup, neurofascin and L1 and their ligands. In contrast to L1, neurofascin is expressed as a complex population of isoforms during development. To analyse the function of this extensive alternative splicing in the extracellular region of neurofascin, we have quantified the binding of different isoforms of neurofascin to its ligands. While the IgSF members NrCAM and F11, as well axonin-1 were found to bind to all isoforms of neurofascin, the ECM component, tenascin-R, interacts only with a subset of neurofascin isoforms. Surprisingly, insertion of short amino acid chains into the neurofascin polypeptide results in a modulation of binding.

The functional consequences of this regulation of binding, by inclusion or omission of specific segments within the neurofascin polypeptide, has been investigated using *in vitro* neurite outgrowth assays. These investigations indicate that neurofascin-mediated neurite extension can be regulated by the presence and binding of interacting proteins.

Disease-associated mutations within the human L1 gene affect heterophilic and homophilic interactions

Sue Kenwrick and Patrick Willems have shown that the L1 protein, which is a key member of this subfamily of IgSF, is involved in an X chromosome-linked human hereditary brain disorder. This disease has been termed X-linked hydrocephalus, MASA syndrome (mental retardation, aphasia, shuffling gait, adducted thumbs) or spastic paraplegia type I. A prominent feature of this disease is a relatively broad spectrum of symptoms which includes mental retardation, lower limb spasticity, hydrocephalus, flexion deformities of the thumbs, hypoplasia of the corticospinal tract and an underdeveloped corpus callosum. Mutations linked to this disease are distributed over all domains of L1, both extracellular and intracellular. As a first step towards understanding the molecular aspects of this disease we investigated how these mutations in the L1 protein influence binding of different ligands, in particular, those ligands which are functionally linked to neurite elongation and fasciculation (in collaboration with S. Kenwrick, Cambridge).

These binding analyses have shown that different disease-associated mutations have distinct effects on heterophilic ligand binding. Several mutations result in a loss or reduction of binding while other mutations lead to increased binding. The heterophilic binding profile of the mutations does not mirror that of homophilic binding. Our findings indicate an involvement of extensive extracellular regions of L1 in interactions with axonin-1 and F11 (see figure). In summary, the fact that pathological mutations can affect either homophilic or heterophilic interactions alone suggests that both forms of L1 binding activity are important *in vivo* and that some aspects of patient pathology are due to disturbances in cell-surface interactions.

Tenascin-R modulates neurite extension on F11 *in vitro*

F11 forms another subgroup of IgSF recognition molecules that delineates subpopulations of axons in the central and peripheral nervous system and is implicated in axonal fasciculation and extension *in vitro*. In contrast to the L1 subgroup of proteins, F11 and the other members of this subclass are anchored to the plasma membrane via a glycosylphosphatidyl inositol (GPI) moiety. F11 appears to exert its function via interactions with multiple heterophilic ligands, including other IgSF members, tenascins and protein tyrosine phosphatases. To gain insight into how these interactions modulate the activities of F11, we have analyzed F11-mediated neurite extension in the presence of tenascin-R or tenascin-C in detail. Our studies indicate that tenascin-R increases cell attachment and neurite outgrowth on immobilized F11. These tenascin-R-induced changes are accompanied by a shift in receptor usage by tectal cells from NrCAM to β 1 integrins. Furthermore, tenascin-R induces morphological changes in tectal neurons including enlargement of growth cones and increased collateral branching of neurites.

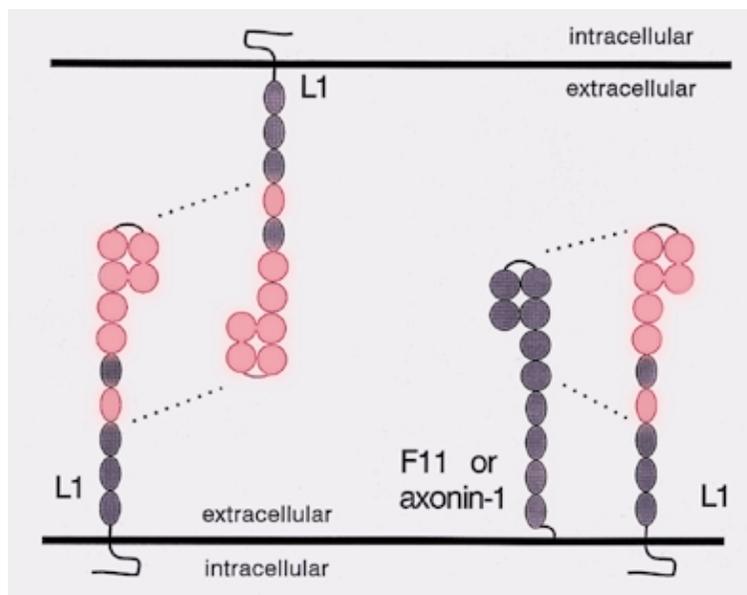
IgLON subfamily: identification of neurotractin

To examine the complex biology of neural IgSF proteins further, we are looking at the identification and functional characterization of novel members of this superfamily. Using a systematic PCR approach, we have identified a novel GPI-linked IgSF member, termed neurotractin, that is expressed on subsets of commissural and longitudinal axon tracts in the developing chick brain. Molecular characterization indicates that neurotractin is a member of the IgLON subgroup of the IgSF which has been created by the limbic system-associated membrane protein implicated in hippocampal circuit formation. Its binding characteristics, histological distribution together with *in vitro* neurite outgrowth studies suggest that neurotractin plays a role in the development of central nervous system axon tracts.

CALEB – a member of the EGF family of differentiation factors in the developing nervous system

Another group of proteins implicated in neuronal differentiation during nervous system development are the members of the EGF family of growth and differentiation factors. By combining binding assays with immunological screening, we recently identified a novel member of this family, which we termed CALEB, and which is expressed exclusively in the nervous system. cDNA cloning indicates that CALEB is a multidomain protein that consists of an N-terminal glycosylation region, a leucine-proline-rich segment, an acidic box, a single EGF-like domain, a transmembrane domain, and a short cytoplasmic stretch. In the developing nervous system, CALEB is associated with glial and neuronal surfaces and is downregulated in the adult nervous system. CALEB binds to the extracellular matrix glycoproteins, tenascin-C and -R, and *in vitro* antibody perturbation experiments indicate the participation of CALEB in neurite formation in a permissive environment.

Figure 41: Schematic representation of the homophilic L1 binding and heterophilic interaction of L1 with F11 or axonin-1. Ig-like domains (circles) and fibronectin-related domains (ellipses) of L1, which carry disease-associated mutations interfering with the molecular interactions, are shown in blue (for details please see De Angelis et al., 1999).



Selected Publications

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As provided by § 7 of its Charter the organs of the Foundation of the Max Delbrück Center for Molecular Medicine (MDC) are the following:

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- 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.

Figure 42 (left): Wolf-Michael Catenhusen from the Federal Ministry of Education and Research, has become Chairman of the Board of Trustees at the MDC at the end of 1998.

Figure 43 (right): Fritz Melchers from the Basel Institute for Immunology (Basel, Switzerland), has shaped the MDC as chairman of the Scientific Committee for almost eight years. He left this post at the MDC in 1999.

Members of the Board of Trustees

Parliamentary State Secretary
Wolf-Michael Catenhusen (Chair)
Federal Ministry of Education and Research, Bonn/Berlin (since March 1999)

Parliamentary State Secretary
Elke Wülfing (Chair)
Federal Ministry of Education, Science, Research, and Technology, Bonn*

State Secretary Prof. Dr. Ingolf Hertel (Vice-Chair)
Senate Administration for Science, Research and Culture, Berlin (since October 1998)

State Secretary Prof. Dr. Erich Thies (Vice-Chair)
Senate Administration for Science, Research and Culture, Berlin*

Dr. Jürgens Behrens
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch, Berlin

Prof. Dr. Hans R. Brunner
C.H.U. Vaudois, Division of Hypertension, Lausanne, Switzerland

Dietmar Bürgener
Federal Ministry of Finances, Bonn/Berlin

Dr. Reinhold Förster
Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch, Berlin

Prof. Dr. Peter Gaegtens
President of the Free University of Berlin, Berlin (since June 1999)

Prof. Dr. Johann W. Gerlach
President of the Free University of Berlin, Berlin*

Dr. Michael Hackenbroch
Federal Ministry of Education and Research, Bonn/Berlin

Prof. Dr. Dr. Falko Herrmann
Institute for Human Genetics, University of Greifswald, Greifswald

Senator Beate Hübner
Senate Administration of Health, Berlin

Prof. Dr. Georg W. Kreutzberg
Max Planck Institute for Psychiatry, Department of Neuromorphology, Martinsried

Prof. Dr. Eckart Köttgen
Director of the Clinical Board of the Charité Medical Faculty of the Humboldt University of Berlin, Berlin

Prof. Dr. Fritz Melchers*
Basel Institute for Immunology, Basel, Switzerland



Prof. Dr. Hans Meyer
President of the Humboldt University
of Berlin, Berlin

Prof. Dr. Mary Osborn
Max Planck Institute for Biophysical
Research, Göttingen
(since October 1999)

Dr. Helmut Schühsler
TVM Techno Venture Management III
GmbH, München (since October 1999)

Prof. Dr. Peter C. Scriba
Inner City Medical Clinic, Munich

Dr. Albert Statz
Federal Ministry of Health,
Bonn/Berlin (since November 1999)

Prof. Dr. Günter Stock
Schering Aktiengesellschaft, Berlin*

Prof. Dr. Volker ter Meulen
Institute of Virology, University
Würzburg, Würzburg (since October
1999)

Prof. Dr. Thomas A. Trautner
Max Planck Institute for Molecular
Genetics, Berlin

Prof. Dr. Ernst-Ludwig Winnacker
Gene center of the Ludwigs
Maximilians University Munich,
Munich*

Dr. Stefan Winter
Federal Ministry of Health, Bonn*

*part of the time reported

Figure 44: Christa Thoben, Senator for Science
and Cultural Affairs at MDC's New Year's
Reception on January 28, 2000.



Members of the Scientific Committee

Prof. Dr. Fritz Melchers (chair)
Basel Institute for Immunology, Basel,
Switzerland*

Prof. Dr. Thomas A. Trautner (chair,
since October 1999)
Max Planck Institute for Molecular
Genetics, Berlin

Prof. Dr. Günter Breithardt
Medical Clinic, University of
Münster, Münster (since April 1999)

Prof. Dr. Hans R. Brunner
C.H.U. Vaudois, Division of
Hypertension, Lausanne, Switzerland

Prof. Dr. Dr. Falko Herrmann
Institute for Human Genetics,
University of Greifswald, Greifswald

Prof. Dr. Georg W. Kreutzberg
Max Planck Institute for Psychiatry,
Department of Neuromorphology,
Martinsried

Prof. Dr. Klaus Müller
Hoffmann-La Roche & Co., Basel,
Switzerland*

Prof. Dr. Mary Osborn
Max Planck Institute for Biophysical
Chemistry, Göttingen (since October
1999)

Prof. Dr. Lennart Philipson
Karolinska Institut, Stockholm,
Sweden (since April 1999)

Prof. Dr. A. Günter Riegger
University Medical Clinic II,
Regensburg*

Dr. Helmut Schühsler
TVM Techno Venture Management III
GmbH, München
(since October 1999)

Prof. Dr. Martin Schwab
Institute for Brain Research,
University Zurich, Zurich,
Switzerland

Prof. Dr. Peter C. Scriba
Inner City Medical Clinic, Munich

Prof. Dr. Kai Simons
European Molecular Biology
Laboratory (EMBL), Heidelberg

Prof. Dr. Günter Stock
Schering Aktiengesellschaft, Berlin*

Prof. Dr. Volker ter Meulen
Institute of Virology, University
Würzburg (since October 1999)

Prof. Dr. Alex J. van der Eb
Department of Molecular
Carcinogenesis, Sylvius Laboratories,
Leiden, The Netherlands

Prof. Dr. Ernst-Ludwig Winnacker
Gene center of the Ludwigs
Maximilians University Munich,
Munich*

*part of the time reported

Figure 45: Gudrun Erzgräber, Business
Director of the BBB Biomedical Research
Campus Berlin-Buch Inc. (3rd from right), and
Erwin Jost, Administrative Director of the MDC
(2nd from left), chatting with a guest at the
MDC's New Year's Reception on January 15,
1999.



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. Dr. Detlev Ganten. Dr. jur. Erwin Jost has been the administrative member of the Management Board.

Scientific Council

According to §14 of the Charter of the MDC, the Scientific Council advises 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 advised the Management Board particularly in matters concerning the establishment of core facilities and the rules and regulations governing permanent employment of scientists. In addition, the Scientific Council made recommendations on working ethics and publication policy, the improvement of the graduate students' education program, as well as on establishing a department for the special requirements of scientists and guests from abroad.

Elections were held in November 1998 and the Scientific Council has been reconstituted.

Members of the Scientific Council
(as of November 1998)

Dr. Martin Lipp (Chair)
Dr. Wolfgang Uckert (Vice-chair)
Prof. Dr. Walter Birchmeier
Prof. Dr. Thomas Blankenstein
Dr. Kurt Bommert
Dr. Iduna Fichtner
Dr. Hannelore Haase
Prof. Dr. Udo Heinemann
Dr. Uta Höpken
Dr. Uwe Karsten
Prof. Dr. Helmut Kettenmann
Dr. Frank Kirchhoff
Dr. Gary R. Lewin
Prof. Dr. Friedrich C. Luft
Dr. Thomas Müller
Dr. Frank W. Pfrieder
Dr. Claus Scheidereit
Prof. Dr. Peter M. Schlag
Dr. Ruth Schmidt-Ullrich
Dr. Gerd Wallukat

Staff Council

The Staff Council at the Max Delbrück Center for Molecular Medicine has a legal right to participate in matters associated with the Center. This includes participating in decisions and collaborating in matters involving employment, grouping, and conversion, 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", "Works-, 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 sub-group for Employment, Professional Training and Women and by the Ministry for Employment, Women, Health and Social Affairs with help from the European Structural Fund (ESF).

Members of the Staff Council 1998/1999

Dr. Dieter Bärwolff
Inge Beyerdörfer
Marion Bimmler (Chair)
Lutz Else
Ingrid Grunewald
Dr. Peter Konzer
Bernd Lemke
Werner Liebig
Christel Westen

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 science, but also the security 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 of safety procedures are all important functions of the safety engineers. The Committee for Working Safety at MDC consists of scientifically trained safety officers and specialists for 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

Renovation of the main working areas in the MDC's buildings was completed by the end of 1998.

Following agreement with donors, an additional working area of 362 m² was constructed in the Max Delbrück House (MDH) for use in connection with GMP activities. This facility allows integration of the basic concepts of quality assurance, good manufacturing practice and quality control of the development and production of sterile drugs.

As far as the planned construction of a new combined facility, intended for animal experimentation and theoretical studies for the Max Delbrück Center (MDC) and the Forschungsinstitut für Molekulare Pharmakologie (FMP), is concerned, agreement on planning the concept has been granted and a date of September 1999 set. The MDC intends to start the project in the year 2000.

Scientific congresses and seminars are key components in raising the national and international profile of the Max Delbrück Center. With this in mind, a Communications Center will be built, incorporating a lecture theatre with a capacity of 500, on the Berlin-Buch Campus.

The building plans have been approved and construction has been scheduled to start in March 2000. The facility is scheduled to be ready for use by October 2001.

Part of the funding for the Communications Center and renovation of the outside of Building 31.1 – MDH – are being met by money from the European Fund for Regional Development (EFRE).

Head: Sören-Peter Plöhn

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 whether laws, practices, regulations and the directives of the Management Board are properly observed and whether allocated public resources are appropriately, economically and productively utilized. For these purposes, examination plans, by approval of the Management Board, are instituted yearly to check for regulatory compliance of organizational activities, such that individual actions are inspected on a case-by-case basis for full propriety with regard to both form and content.

All checks of regulatory compliance are undertaken with respect for economic soundness and productiveness, so as to ensure that proper judgment is exercised in establishing regulations and in the execution of business matters. These checks result in the announcement of recommendations as to how detected oversights might best be alleviated 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 partners in Industry. In addition, jurisdictional proceedings must be prepared, and in many individual cases legal consultation must be provided.

Head: Christine Rieffel

Patents/Licences

The total number of German patents and patent applications from the MDC in 1999 amounted to 98, compared with 86 in 1998 and this included 3 patents which had been awarded before the MDC was established. In 1999, for the first time, the annual number of patent applications reached 20, compared with 19 in 1998. This shows that there is no sign of the innovative ability of MDC researchers reaching a plateau.

The first two US patents for discoveries originating from the MDC were awarded in 1999. There were also 12 PCT (Patent Cooperation Treaty) patent applications. In 1999, 3 contract options and 2 licensing contracts were awarded. In addition, the first sale of an MDC patent was concluded. The license income for 1999 amounted to 145,207 DM, compared with 111,711 DM for 1998. This represents a significant year-on-year increase.

The MDC has set up a Legal Protection Committee with Dr. Iduna Fichtner, Prof. Walter Birchmeier and Dr. Martin Lipp as members. It is the responsibility of this committee to investigate whether application should be made for foreign patents, in addition to the primary patent protection sought in Germany. This involves the inventors submitting an application to the MDC board and, following a checking procedure by the attorney, Dr. Fritz Baumbach, it is then submitted to the Legal Protection Committee for their consideration.

Head: Dr. Fritz Baumbach

Technology Transfer

The MDC strongly supports all activities concerning the potential commercialization of research results. Therefore, a Technology Transfer Committee headed by Iduna Fichtner has been set up to manage and optimize all activities and improve cooperation among research institutes, small and medium enterprises (SME), and the clinics.

In recent years, these activities have led to an increased number of filed and approved patents and this process has been markedly improved by external evaluation of submitted manuscripts for patentable research findings before publication. Activities involving the commercialization of patents have been intensified by direct contact with licensing agencies (e.g. Fraunhofer Patentstelle, British Technology Group). In addition, a Technology Transfer Conference has been held to give scientists the opportunity for direct contact with potential sponsors for their innovations. This conference will be held once a year.

Within the MDC, a "Biomedical Research Transfer" initiative has been set up comprising several groups with long-standing and successful experience in applied research. The process of technology transfer will be facilitated by mutual support in grant applications, intensified cooperation and joint presentation of scientific results.

Since 1997, six companies have been founded on the campus Berlin-Buch by outsourcing of scientific results from the MDC. Among them are ATUGEN AG, developed by a joint venture with Ribozyme, GenProfile AG, involved in the search for disease-related genes, Kelman GmbH, focussing on the prediction of protein-ligand interactions and EPO GmbH, offering support in the development of novel anticancer agents.

A joint venture with Schering AG recently led to the foundation of GTB Gene Therapeutics, engaged in the GMP-certified production of viral and nonviral vectors.

Head: Dr. Iduna Fichtner



Figure 46: Ceremony laying the foundation stone for the second new laboratory building of the BBB's Biotechnology and Business Development Center for start-up companies on December 17, 1999 with Wolfgang Branoner, Berlin Senator for Economic Affairs (on the left), Detlev Ganten, Scientific Director of the MDC, and Gudrun Erzgrüber, Business Director of the BBB Biomedical Research Campus Berlin-Buch GmbH (front row, from left to right).

Press and Public Relations

Research at the MDC is conducted at the forefront of biomedical science - in the promising 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 MDC's expenses must be justified to the German tax payer. The Press Department initiated various activities to serve this need which are outlined below.

In 1998 and 1999 the MDC Press Department 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 laboratories of the MDC, the cooperating university affiliated clinics, Robert-Rössle-Cancer-Clinic and Franz Volhard Clinic for Cardiovascular Diseases, and the Hands-on Laboratory at the Biomedical Research Park.

In 1998 and 1999 the MDC Press Department also continued the series of popular scientific lectures in the City Hall of Berlin Pankow, initiated in 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 MDC's research at the Hannover Industrial Fair and at the Berlin Science Fair.

A total of 43 news releases published in German and English by the MDC Press Department in 1998 and 1999 were the basis for many reports in the media. About 2 000 newspaper articles with a circulation of well over 230 million copies were published on the MDC, the Robert Rössle Clinic and Franz Volhard Clinic, and the Biomedical Research Park. In addition, 16 television and 16 radio productions, including the BBC Tomorrow's World and Arte, the French/German program, were aired on the research conducted at the MDC, the clinics and the Biomedical Research Park.

In the time-span reported, three press conferences were initiated, organized and moderated by the MDC Press Department. One of these press conferences was held at the "6th International Gene Therapy Symposium" in Berlin-Buch in 1998, the other at the Grand Opening of the Biotechnology Business and Development Center of the BBB Biomedical Research Campus GmbH, also in 1998. The third press conference was initiated to inform about genome research with the American Nobel laureate Paul Berg. He had given a talk in the series of the "Berlin Lectures on Molecular Medicine" in 1999 and had received the Max Delbrück Medal of the MDC and other Berlin research institutions, and the Schering Forschungsgesellschaft at that occasion.

In 1998 and 1999 the MDC Press Department prepared and organized more than 160 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. It also published four press reports summarizing the coverage of the MDC, the clinics, the Biomedical Research Park, and related topics in the printed press as well as two issues of the MDC-Report, an in-house magazine.

Head: Barbara Bachtler



Figure 47: The MDC is an international research institute, attracting scientists from all over the world

Administration

Personnel Department

The department is responsible for all matters relating to staff, wages, salaries, separation allowances, removal and travel expenses etc.

During the last eight years, MDC has not only managed to function efficiently as a unified body, but staff also work in close harmony. During 1992, a total of 382 staff were employed by MDC and, by December 1999, this figure had risen to 653, including those (199) paid by third-party funding. As before, most (89 %) of the scientists' contracts are limited to a maximum of five years.

MDC is currently financing 30 graduate students studying for a PhD, who are not included in the list of employees. In addition, at MDC, there are 68 part-time, third-party financed, young scientists and 2 graduate students studying for a PhD, who are also third-party financed.

Head: Dr. Hans-Joachim Seehrich

Finances

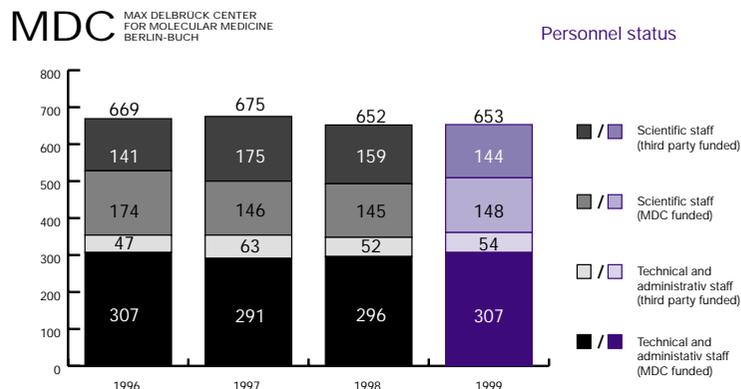
The Finance Department concerns itself with all matters relating to MDC's financial funding, including accounting. The primary source (90 %) of MDC's annual funding comes from the Federal budget (Federal Ministry of Education and Research). The remaining 10 % is provided by the State of Berlin (Senate Administration for Science, Research and Culture). Within the framework of its basic funding, MDC will receive 99 million DM for the year 2000; approx. 18 million DM (as of December 1999) will be made available in 2000 from third-party financial sources.

Increases have also taken place in the staff sector, due to general wage rate increases and, in particular, increases within Berlin. A decline in spending is seen in investments in equipment after a period of heavy investment during MDC's first years. Extensive measures are necessary to maintain the MDC laboratories in their present structural state. Another positive development has been in attracting third-party financial resources and, in 1996, the MDC was able to spend 17 million DM of third-party money.

As mentioned above, approval for approx. 18 million DM of extra-mural funding has already been received for the year 2000.

Head: Wolfgang Kühlewind

Figure 48: Personnel status.
Distinctions according to financial sources.



Purchasing and Materials Management

The tasks of the Purchasing and Materials Management Department are focused on three main areas:

- rapid and efficient supply of quality laboratory materials, auxiliary and consumables, 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 17,700 orders are processed yearly. Compared to MDC's early years, this is an increase of 2 percent. In particular, the number of orders for chemicals has risen (about 4 percent).

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 their orders themselves. This will guarantee fast processing in purchasing. Linking the scientist's 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

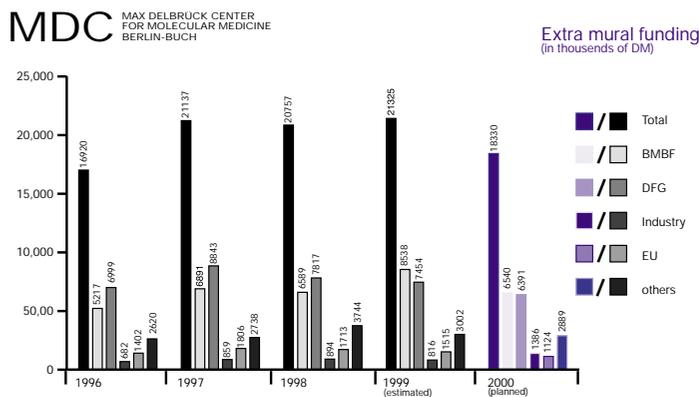


Figure 49: Approved third-party financed projects and other financial funds.

Third-party funds are represented according to their individual sources, the 2000 estimate is based on the scope of approvals received, dated December 10, 1999. BMBF = Federal Ministry of Education and Research; DFG = Deutsche Forschungsgemeinschaft.

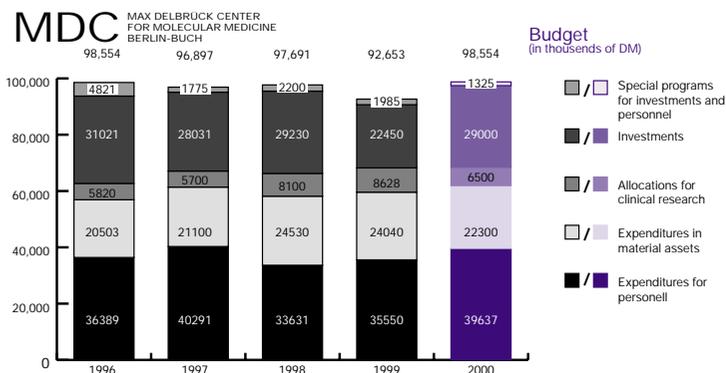


Figure 50: MDC's budget development in individual expenditure sectors between 1996 and 2000 (estimated).

Central Facilities

Library

The MDC library is a specialized scientific library. Its work involves providing and supplying information in all research areas of the MDC and its associated clinics. The library acts as a modern information centre using advanced network structures and allowing access to information sources around the world. Modern information networks offer the increased possibility of supplying users with specific literature and information resources at their work place. The collection contains more than 40,000 media items and 280 periodicals, many of which are additionally provided in electronic form, and different kinds of non- print materials. Campus-wide provision of major local databases include Medline (starting from 1966), Current Contents (5 Science Editions) as well as Science Citation Index with abstracts (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, 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 service 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 at 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. Animal experiments are especially concerned with the development of methods for improved diagnosis and treatment of human tumors and cardiovascular diseases. They are essential for 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 to allow the production of transgenic rats and mice and mouse chimeras. Since then, more than 70 transgenic rat strains have been developed to model hypertension. In addition, mice have been reconstructed by ES-cell injection and have been successfully bred since 1995. More than 250 strains of knock-out mice are now available as experimental models in cancer research, as well as cardiovascular and neurological diseases. Of all the research institutes in Berlin, the animal facility of the MDC has the greatest number of genetically engineered rat strains and mice stocks.

Four animal houses support transgenic and animal experimentation at the MDC and mice, rats and rabbits are bred. Occupying 1,260 square meters, the facilities include animal rooms (636 m²), operating theatres, storage rooms and cage-washing facilities and 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 meet present requirements.

The rapidly growing number of genetically engineered models of severe human illness means that further animal facilities for breeding and experimentation are needed. The MDC, therefore, plan to construct a new central animal house. The project has been approved by the Board of Trustees and will, hopefully, be opened by the year 2003.

Head: Dr. Karin Jacobi

Campus Net Management

The Campus Net Management of the BBB Biomedical Research Campus Berlin-Buch GmbH is responsible for the operation of the high-speed campus network to supply of campus-wide internet server services (mail-, WWW-, FTP-, and phone-servers), and for communication with other networks, e.g. B-WiN (Science Net) and BRAIN (Berlin Research Area Network). Further services for the Campus, like video-conferencing and billing/accounting are under construction.

Head: Hans Mitulla

Figure 51: The sculpture "Großer Nagelkopf" by the artist Rainer Kriester was exposed on the Berlin-Buch Campus from September 1998 to February 1999 as part of a changing exhibition of sculptures, expressing the link between science and art on the Campus.



Computing

The computing group of the MDC manages the central computer facilities of the MDC (Remote Access-, File-, and Backup-servers).

The group is responsible for the client/server operation of the MDC's Administration and Executive Board, and is responsible for the system- and user-support of the SAP-R/3-administration system.

The group focuses its activities on user-oriented support of data and image processing at the MDC. The group 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 usage both the central computer facilities of the MDC and the campus-wide computer facilities of the BBB GmbH. In addition, the group organises standard software courses in our computer laboratory, which is equipped specifically for such purposes.

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.

Head: Bernd Lemke

Technical Affairs

During the period covered by this report, the Technical/Works Department has carried out key work associated with the takeover of technical operations for the GMP sector.

In conjunction with this, a number of technical safety systems have been installed, functions checked and reflected in the Latest Building Techniques (GLT). In the research buildings of the MDC, air-conditioning equipment has been widely installed in the areas that have undergone reconstruction and has now been taken over from the Technical/Works Department. Here, too, the switch to GLT was of particular importance.

The telephone equipment of the MDC has been upgraded to a highly sophisticated level and a voice mail system has been added.

This now means that the telephone equipment meets ISDN standards. All MDC buildings have been fitted with modern fire alarms and the building are linked to one another and connected to the Berlin Fire Department *via* a CSN computer.

Head: Harry Schenk

Figure 52: Walter Friedrich House, laboratory building of the MDC. The building is named after Walter Friedrich, Head of the Institute of Medicine and Biology of the Academy of Sciences in Berlin-Buch from 1948 to 1960. He was renowned for his contributions with Max von Laue to the electromagnetic nature of X-rays and to X-ray crystallography of proteins and their structure.



Meetings, Workshops and Symposia

The following events organized under the auspices of the MDC and its clinical partners took place in 1998 and 1999

Staging Laparoscopy
(March 20-21, 1998)

4th MDC Graduate Students' Symposium
(March 26, 1998)

6th Symposium on Gene Therapy "Towards Gene Therapeutics"
(May 4-6, 1998)

2nd Congress of Molecular Medicine
(May 6-9, 1998)

Forum of European Neuroscience
(June 27 - July 1, 1998, Inter-Continental, Berlin)

2nd Cell Biology Symposium of the MDC: Protein Transport and Stability
(September 5-9, 1998)

120. Jahrestagung der Gesellschaft Deutscher Naturforscher und Ärzte
(September 19-22, 1998, Humboldt University, Berlin)

Jahrestagung der Hermann-von Helmholtz-Gemeinschaft Deutscher Forschungszentren
(November 4-5, 1998, Humboldt Universität zu Berlin)

MDC Symposium Molecular Medicine
(December 18-19, 1998)

Bucher Symposium "Molecular Genetics and Genome Analysis"
(March 4, 1999)

5th MDC Graduate Students' Symposium
(March 10, 1999)

Berliner Seminar "Moderne Peptid- und Proteinanalytik: Anwendungen und Limitierungen"
(March 19, 1999)

9th European Congress of Clinical Microbiology and Infectious Diseases
(March 21-24, 1999, ICC, Berlin)

Application of Molecular Methods for the Development of New Therapies
(March 29-31, 1999)

Informationsveranstaltung zum 5. EU-Rahmenprogramm für Forschung, technologische Entwicklung und Demonstration (1998-2002)
(March 31, 1999)

BIO'99
(May 16-20, 1999, Seattle/USA)

Zukunft Biotechnologie
(May 28, 1999)

Molekulare Kardiologie: Neue Forschungsstrategien gegen Herzinsuffizienz
(August 27, 1999)

Solution Structure and Interaction of Biopolymers using Analytical Ultracentrifugation (October 19, 1999)

Berlin Lectures on Molecular Medicine with Prof. Paul Berg, Stanford University, School of Medicine, Beckman Center for Molecular and Genetic Medicine, Stanford, USA
(November 18, 1999 Charité Medical Faculty of the Humboldt University of Berlin)

1st Technology Transfer Conference in Berlin-Buch
(November 22, 1999)

Jahrestagung der Hermann-von Helmholtz-Gemeinschaft Deutscher Forschungszentren
(November 24-25, 1999, Bonn)

3rd Congress of Molecular Medicine (CMM) and VIth Franz-Volhard-Symposium "Molecular Mechanisms in Dilated Cardiomyopathy"
(December 3-4, 1999)

Michael-Strauss Memorial Lecture, Jeffrey Leiden (Harvard School of Public Health, Boston/USA), "Genetic Approaches to Understanding and Treating Heart Failure" at the 3rd Congress of Molecular Medicine (CMM) and VIth Franz-Volhard-Symposium "Molecular Mechanisms in Dilated Cardiomyopathy"
(December 4, 1999)

Figure 53: Xu Zhihong (3rd from left), Vice-President of the Chinese Academy of Sciences (CAS), in a meeting with Detlev Ganten, Scientific Director of the MDC (2nd from left), and Chinese guest scientists at the MDC on the occasion of his visit to Berlin-Buch in November 1998.



Awards

Thomas Biederer
Boehringer-Mannheim-Förderpreis
der Deutschen Gesellschaft für
Zellbiologie, 1998

Jens Reich
Urania-Medaille, 1998

Regina Reszka
Innovationspreis des Landes Berlin-
Brandenburg, 1998

Thomas E. Willnow
Heinrich-Wieland-Preis, 1998

Jürgen Behrens
Monika Kutzner-Preis zur Förderung
der Krebsforschung der Berlin-
Brandenburgischen Akademie der
Wissenschaften, 1999

Walter Birchmeier,
Peter M. Schlag
Deutscher Krebspreis 1999

Hermann Haller,
Volker Homuth,
Friedrich C. Luft,
Gerd Wallukat
Galenus-von-Pergamon-Preis, 1999

Max-Delbrück-Medal

Since 1992, outstanding scientists are being awarded the Max Delbrück Medal by Berlin research institutions and the Schering Research Foundation. In 1998, the Swedish anthropologist Svante Pääbo received the Max Delbrück Medal, and in 1999, the American Nobel-Laureate Paul Berg (Stanford University, California). In 2000, this medal has been awarded to Fritz Melchers (Basel Institute for Immunology), head of the Scientific Committee of the MDC till 2000. Recipients in the past years were:

1992 Günter Blobel
(Rockefeller University New York ,
USA; Nobel Laureate in 1999)

1994 Sydney Brenner
(University of Cambridge, UK)

1995 Jean-Pierre Changeux
(Institut Pasteur, Paris, France)

1996 Robert A. Weinberg
(Whitehead Institute, Massachusetts
Inst. of Technology Cambridge, USA)

1996 Nihat Bilginturan
(University of Hacettepe, Ankara,
Turkey)

1997 Charles Weissmann
(University of Zürich, Switzerland)

1998 Svante Pääbo
(Ludwig-Maximilians-Universität
München and Max Planck Institute for
Evolutionary Anthropology, Leipzig)

1999 Paul Berg
(Nobel-Laureate 1980; Stanford
University, California)

2000 Friedrich Melchers
(Basel Institute for Immunology,
Basel/Switzerland)



Figure 54: Nobel Laureate Paul Berg from Stanford University, California, USA (in the middle), on his visit to the Berlin-Buch Campus on November 18, 1999 with MDC-scientist Martin Lipp and Stefanie Korthals.

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Index

A

Abdul, Yetunde 90
Aguirre-Arteta, Ana M. 88
Ahlers, Annette 68
Al Kateb, Hussam 35
Al-Yahyaee, Said Ali 35
Alenina, Natalia 23
Alexander, Gabriele 27, 96, 97
Ambrosino, Concetta 43
Ameln, Ingrid 83
Andrade, Miguel 43
André, Françoise 29
Andréé, Christel 94
Andreev, Dmitri 23
Ansieau, Stephane 66
Antonius, Mike 122
Anzinger (nee Panzenböck), Birgit 119, 120
Appel, Silke 29
Arndt, Dietrich 102, 121, 160
Asbrand, Christian 75, 77, 79
Aumann, Jutta 109
Axmann, Guido 23
Ay, Jacqueline 50, 52
Aydin, Atakan 35, 43

B

Babu, Allampura M. 50, 52
Bachtler, Barbara 145, 160
Bader, Michael 18, 22, 23, 85, 160
Badock, Volker 60, 103
Bähring, Sylvia 35
Baldy, Christina 109
Baltas, Leonidas 85
Baltatu, Ovidiu 23
Balzereit, Daniela 85
Barbosa-Sicard, Eduardo 94
Bargou, Ralf 72, 74, 103, 104, 105
Baron, Heike 35
Bartel, Sabine 82, 83
Barthel, Beatrix 31, 32
Bartunek, Petr 119, 120
Bärwolff, Dieter 122, 142
Bauer, Lisa 90
Baumbach, Fritz 144
Becker, Christin 23, 107
Becker, Michael 109
Becker, Monika 109
Becker, Christian 117
Becker, Katja 117
Becker-Follmann, Johannes 29
Begay, Valerie 66
Behlke, Joachim 19, 44, 45, 49, 54, 99, 160
Behrend, Martin 77
Behrens, Diana 109
Behrens, Jürgen 13, 15, 62, 73, 75, 77, 78, 79, 89, 140, 151, 160
Bengs, Marion 66
Berger, Ingrid 15, 112
Bergmann, Jutta 112
Bergmann, Stephan 73, 74
Bernhardt, Günter 71
Beyerdörfer, Inge 83, 142
Bienert, Ralf 52
Bieringer, Markus 27
Bigott, Jessica 107
Bimmler, Marion 142, 160
Binas, Bert 23
Birchmeier, Carmen 18, 19, 23, 36, 37, 75, 76, 77, 160
Birchmeier, Walter 15, 63, 75, 77, 78, 79, 89, 99, 142, 144, 151, 160
Bischof, Dana 41
Blachut, Susanne 21

Blankenstein, Thomas 8, 13, 102, 106, 107, 116, 117, 142, 160
Blendinger, Gitta 119, 120
Boeckh, Susanne 123, 124
Böckel, Thomas 35
Bödner, Bärbel 45
Böhm, Siegfried 56
Boetzel, Joachim 60
Bohlender, Jürgen 20, 21, 27, 82
Bommert, Kurt 59, 60, 72, 74, 103, 105, 142
Bönsch, Christian 41
Bork, Peer 19, 35, 43
Bosnar, Martina 90
Böttger, Adelheid 23
Boucsein, Clemens 130
Brachwitz, Hans 160
Brand, Karsten 74, 124
Braun, Oliver 112
Breitfeld, Dagmar 71
Brett, David 35, 43
Brinkmann, Volker 37, 75, 77
Britsch, Stefan 36, 37
Brohmann, Henning 37
Brümmendorf, Thomas 137
Buchert, Sven 37
Burkhardt-Göttges, Elke 29, 31, 32
Burgstahler, Ralf 71
Burmeister, Regina 41
Busjahn, Andreas 25, 34, 35
Busjahn, Dorothea 148, 160
Butschak, Günter 80, 81

C

Calkhoven, Cor 66
Cardoso, M. Cristina 86, 88, 113
Cartier, Regis 90
Carvalho Araujo, Ronaldo de 23
Catenhusen, Wolf-Michael 140, 160
Cayeux, Sophie 105, 107, 117
Cayla, Cécile 23
Cesare, Silvana Di 77
Chaboissier, Marie-Christine 39
Chai, Guixuan 23, 85
Choli, Theodora 60
Cichon, Günther 123, 124
Cifire, Felix 71
Claßen, Elvira 30, 32
Cloos, Birgit 52, 137
Contzen, Jörg 54
Czychi, Jana 97

D

Daemen, Kerstin 107
 Dakos, Vassilios 90
 Damaschun, Gregor 19, 46, 47, 48, 160
 Damaschun, Hilde 47
 Dandekar, Thomas 43
 Daniel, Peter T. 59, 103, 105, 107
 Danneberg, Heike 23
 Debs, Pierre 72
 Dehapiot, Elyzabeth 54
 Dehmel, Bastian 27
 Dehmel, Anke 109
 Delbrück, Sebastian 25, 35, 43
 Delbrück, Heinrich 50, 52
 Dell'Oro, Andrea 75, 77
 Derer, Wolfgang 88
 Dettmer, Rudolf 68
 Diebold, Sandra S. 119, 120
 Diederichs, Julia Eva 112
 Diehl, Annette 50, 52
 Dietz, Rainer 8, 29, 35, 87, 88, 102, 113, 160
 Dong, Jiaxin 98
 Doerks, Tobias, 43
 Dörken, Bernd 8, 59, 68, 72, 73, 74, 102, 103, 105, 107, 117, 160
 Dorner, Armin 137
 Drab, Marek 97
 Drechsler, Hannelore 137

E

Easwaran, Hariharan P. 88
 Eckardstein, Kajetan von 112
 Egorov, Tsezi 60
 Eilers, Andreas 132
 Eisenhaber, Birgit 43
 Eisenhaber, Frank 43
 Eleskurtay, Sefer 124
 Else, Lutz 142
 Emmerich, Florian 68, 74, 103, 105
 Engel, Felix 113
 Engel, Katrin 72, 112
 Epps, Amanda von 83
 Erdenechimeg, Chimge 27
 Erdmann, Bettina 75, 77, 93, 99, 100
 Esslinger, Christoph 120
 Eßmann, Frank 60
 Estevéz-Schwarz, Lope 30, 32, 89, 90

F

Faass, Bettina 60
 Fabian, Heinz 19, 48, 49, 160
 Fädrieh, Birgit 29
 Fälber, Katja 49, 51, 52
 Falk, Kirsten 13, 118, 160
 Faulhaber, Hans-Dieter 25, 34, 35
 Feldner, Anja 21
 Feller, Katharina 79
 Feske, Anette 52
 Fichtner, Iduna 90, 102, 108, 109, 121, 142, 144, 160
 Fiedler, Dora 126
 Fischer, Uta 124
 Flachmeier, Christina 25
 Fleischhauer, Silke 130
 Förster, Alexandra 29
 Förster, Reinhold 13, 71, 140
 Franke, Gudrun 90
 Franke, Renate 76, 77
 Frege, Renate 32
 Friedlander, Ruth 91, 92
 Fürst, Robert 43
 Fujita, Yasuyuki 77
 Fürstenau, Michael 96, 97

G

Gaertner, Klaus 122
 Gaio, Ursula 36, 37
 Gaiser, Olaf 50, 52
 Galat, Vassili 23
 Gallagher, Irene 120
 Galle, Renate 32
 Ganten, Detlev 6, 12, 18, 20, 21, 23, 26, 142, 160
 Ganten, Ursula 85, 100
 Ganzel, Karin 68
 Garratt, Alistair 36, 37
 Gärtner, Angelika 117
 Gast, Klaus 47, 48, 49
 Gaudet, François 88
 Geheeb, Martina 124
 Gehring, Niels H. 76, 77
 Geißler, Erhard 160
 Genersch, Elke 97
 Gerlach, Brigitte 130
 Gibson, Meino Alexandra 130, 152
 Gladow, Monika 117
 Goan, Silvia-Renate 109
 Goldbrich, Beate 124
 Goletz, Steffen 80, 81
 Gollasch, Maik 80, 96, 97
 Gong, Maolian 21
 Göritz, Christian 134
 Gösele, Claudia 21
 Gossen, Manfred 13, 72, 160
 Gottschling, Karin 37
 Gräler, Markus 71
 Gras, Christiane 130
 Grasczew, Georgi 90
 Grasmö-Wendler, Unn-Hilde 31, 32
 Graßhoff, Peter 71
 Grelle, Gerlinde 60, 74
 Grieben, Marlies 113
 Grigull, Sabine 90
 Grimm, Jan 77, 79
 Grinstein, Edgar 74
 Grishkovskaya, Irina 51, 52
 Grittner, Christine 80, 81
 Gromelski, Sandra 112
 Gross, Volkmar 26, 27, 87, 93, 94
 Großmann, Katja 85
 Groth, Detlef 15, 112
 Grüger, Sabine 27
 Grunewald, Ingrid 88, 142
 Gunia, Wilfried 29
 Guo, Jiankan 39
 Gust, Tatjana C. 120

H

Haase, Hannelore 85, 97, 100, 142
 Haase, Sabine 90
 Haefker, Antje 105
 Haensch, Wolfgang 89, 90
 Hagemann, Dirk 83
 Hahn, Mike 90
 Hahn, Stefanie 30, 32
 Haïnk, Petra 120
 Haller, Hermann 13, 15, 26, 27, 41, 93,
 95, 96, 97, 98, 113, 115, 151, 160
 Hammes, Annette 39
 Hampig, Franziska 27
 Hanisch, Uwe-Karsten 130
 Hanke, Jens 42, 43
 Harder-d' Heureuse, Johanna 29
 Harsdorf, Rüdiger von 87, 88, 113
 Hartmann, Gudrun 77, 112
 Hatada, Eunice 68
 Hauck, Ludger 88, 113
 Hauke, Lisa 29
 Haupt, Irene 134
 Häusler, Georg 130
 Heere, Petra 25
 Heikenwälder, Mathias F. 119, 120
 Heinemann, Udo 19, 31, 50, 52,
 142, 160
 Heißmeyer, Vigo 68
 Hemme, Jens 117
 Hempel, Petra 83
 Hennies, Hans Christian 29
 Henning, Mechthild 137
 Heppenstall, Paul 132
 Heringer-Walther, Silvia 33
 Hess, Dana 23, 39
 Hieke, Brigitte 21
 Hildebrandt, Kati 105
 Hildenbrand, Ulrich 83
 Hilpert, Jan 41
 Hinz, Britta 29
 Hinz, Michael 68, 123
 Hirano, Fuminori 68
 Hjerrling-Leffler, Jens 134
 Hoch, Brigitte 82, 83
 Hoehe, Margret 19, 24, 25, 34, 35, 160
 Hoffmann, Anja 130
 Hoffmann, Katrin 29, 34, 35
 Hofmann, Wera 30, 32
 Hofstätter, Maria 66
 Hohaus, Annette 85, 100
 Höhnemann, Dirk 105
 Homuth, Volker 15, 27, 96, 97,
 115, 151
 Honeck, Horst 94
 Hong, Liu 21
 Höpken, Uta 71, 142
 Horn, Denise 30, 32
 Hortsch, Michael 137
 Hübner, Norbert 20, 21, 39
 Huijnen, Martijn 43
 Hülsken, Jörg 75, 76, 77, 99
 Hummel, Kordelia 124
 Hun Lee, Kang 106, 107

I

Ibe, Sabrina 117
 Ibikli, Adnan 121
 Isac, Corina-Mihaela 85
 Ivanov, Anton 60
 Ivics, Zoltán 13, 102, 125, 126, 160
 Izsvák, Zsuzsanna 126

J

Jacobi, Karin 148, 160
 Jäger, Katrin 73, 74
 Jan, Guillaume 54
 Jandrig, Burkhard 30, 31, 32
 Janke, Jürgen 32
 Janta-Lipinski, Martin von 97, 122
 Janz, Martin 73, 74
 Jarchow, Birgit 130
 Jarosch, Ernst 91, 92
 Jeblonski, Frank 71
 Jerchow, Boris 78, 79
 Jia, Wentao 74
 Jobsky, Dieter 137
 Johnen, Heiko 71
 Jorcke, Dierk 60
 Jordan, Jens 8, 26, 27
 Joschko, Simone 66
 Jost, Erwin 142, 160
 Jost-Reuhl, Brigitte 109
 Ju, Xin-Sheng 119, 120
 Jundt, Franziska 74, 103, 105
 Jung, Bories 29
 Jung, Martin 29
 Jung, Christiane 19, 53, 54, 160
 Jungblut, Peter 60
 Junghahn, Ilse 109
 Junghans, Christine 35
 Jungmann, Sabine 68
 Jürchott, Karsten 73, 74, 89, 90

K

Kaba, Rudolf Gabor 71
 Kagelmaker, Horst 130
 Kaiser, Frank 106, 107
 Kammertöns, Thomas 117
 Kanehl, Anke 132
 Kang, Ningling 23, 26, 27
 Kannen, Brunhilde 58
 Karawajew, Leonid 104, 105
 Karczewski, Karin 115
 Karczewski, Peter 82, 83
 Kärgel, Eva 94, 100
 Karsten, Uwe 80, 81, 89, 90, 142, 160
 Katzer, Andrea 105
 Kemmner, Wolfgang 80, 89, 90
 Kemsies, Christel 85
 Kettenmann, Helmut 13, 128, 129,
 130, 142, 152, 160
 Kettritz, Ralf 95, 97
 Keyner, Daniela 68, 71
 Khier, Harald Horst 137
 Kirchhoff, Frank 130, 142
 Kirsch, Thorsten 97
 Kirsch, Frank-Peter 137
 Kistel, Heide 21
 Klahn, Sylvia 92, 117

Klamm, Ursula 85
 Klautza, Jeanette 29
 Klein, Eirien 35
 Klein, J. 60, 97
 Klein, Nicolai 90
 Klein, Rüdiger 131, 132
 Klewer, Jacqueline 134
 Knespel, Andreas 52
 Knespel, Siegne 119, 120
 Knoblauch, Margit 21
 Knoblauch, Hans 35, 43
 Knoblich, Maria 98
 Koberling, Annemarie 81
 Koch, Ina 43
 Köhler, May-Britt 94
 Köhler, Matthias 95, 97
 Kohrt, Christiane 81
 Köble, Konrad 31, 32, 89, 90
 Köpke, Karla 25
 Konzer, Peter 142, 147, 160
 Kordes, Uwe 68
 Koritschoner, Nicolas P. 119, 120
 Körner, Ida-Johanna 105
 Koroll, Michael 137
 Kostka, Susanne 60, 85, 100
 Kotitschke, Erika 100
 Kott, Monika 85, 100
 Kowenz-Leutz, Elisabeth 62, 66
 Krafft, Christoph 58
 Kraft, Regine 60, 73, 74, 85, 100
 Kramer, Annett 57, 81
 Krämer, Jochen 88
 Krappmann, Daniel 68, 74, 105
 Krause, Ernst-Georg 82, 83, 160
 Krause, Julia 29
 Krause, Sabine 66
 Kresse, Wolfgang 130
 Kries, Jens-Peter von 75, 77
 Kröber, Reinhard 47
 Krüger, Kerstin 105
 Krukenberg, Ina 105
 Krusekopf, Solveigh 94
 Kühlewind, Wolfgang 146, 160
 Kuhle, Verona 83, 85, 115
 Kuhn, Susanne 130
 Kulbe, Hagen 71
 Kuring, Dirk 29
 Kurz, Steffen M. 119, 120
 Kurzchalia, Teymuras 160
 Kusch, Angelika 71
 Küttner, Irmgard 117

L

Lamounier-Zepter, Valeria 85
 Landrock, Danilo 39
 Lang, Mathias 112
 Lange, Christian 90, 117
 Lange, Robert 71
 Langenickel, Thomas 23
 Lankes, Wolfgang 36, 37
 Lathe, Warren 43
 Lättig, Stefan 58
 Lauble, Hanspeter 50, 52
 Lee, Young-Ae 20, 28, 29
 Leheste, Jörg-Robert 41
 Lehmann, Cathleen 112
 Lehmann, Christine 122
 Lehmann, Gerrit 35, 43
 Leistner, Werner 56
 Lemke, Bernd 142, 149, 160
 Lemke, Britt 119, 120
 Lemm, Margit 109
 Lenk, Uwe 91, 92
 Lenter, Klaus-Ulrich 25
 Leonhardt, Heinrich 63, 86, 88,
 113, 160
 Leutz, Achim 62, 63, 64, 66, 160
 Lewin, Gary R. 128, 131,
 132, 142, 160
 Li, Guo-Hua 25
 Li, Li 37
 Li, Liang-Ping 117
 Li, Pei-Feng 113
 Liang, Han 90
 Liebig, Werner 142
 Liebner, Iska 29
 Lin, Shaoqian 90
 Lindner, Tom 34, 35
 Lindschau, Carsten 85, 96, 97, 98,
 100, 115
 Linkens, Hans-Josef 160
 Lipp, Martin 69, 71, 142, 144, 160
 Liu, Songjie 25
 Lode, Jörg 112
 Löffler, Anja 104, 105
 Löhn, Matthias 96, 97
 Lucius, Hans 132
 Ludwig, Wolf-Dieter 104, 105
 Luft, Friedrich C. 8, 15, 18, 19, 20,
 25, 26, 27, 28, 35, 41, 42, 43, 60,
 93, 94, 96, 97, 115, 142, 151, 160
 Lutsch, Gudrun 85, 99, 100

M

Maasch, Christian 96, 97
 Madruga, Jaime 119, 120
 Maetzig, Martin 49
 Maier, Alexandra 56
 Maier, Olaf 98
 Malcherek, Lieselotte 90
 Manasterski, David 29
 Mannsfeldt, Anne 131, 132
 Mapara, Markus 74, 105, 119, 120
 Marg, Andreas 137
 Margot, Jean B. 88
 Mathas, Stefan 68, 103, 105
 Matthes, Eckhart 102, 122, 160
 Matyash, Marina 130
 Matyash, Vitali 130
 Mauch, Daniela 134
 McIlwrath, Sabrina 132
 Meding, Jörg 88
 Meese, Carmen 71
 Mehrhof, Felix 68, 88, 113
 Meißner, Kathrin 21
 Mennier, Ulrike 29
 Menzel, Ralph 94, 100
 Mertsch, Katharina 130
 Metzner, Sylvia 74, 105
 Meusser, Birgit 92
 Meyer, Dagmar 71
 Meyer, Mirjam 119, 120
 Micheel, Burkhard 13, 31, 32
 Milia, Anna Franca 26, 27
 Misselwitz, Rolf 58
 Mitulla, Hans 148
 Möhring, Regina 143, 160
 Mohaupt, Mariette 117
 Mokros, Thilo 71
 Moldenhauer, Ferdinand 43
 Molweide, Andreas 106, 107
 Montag, Christina 124
 Monti, Jan 21
 Morano, Ingo L. 13, 84, 85, 97,
 100, 160
 Mordmüller, Benjamin 68
 Moré, Margret 137
 Morkel, Markus 77
 Morwinski, Rosemarie 115
 Mouro, Corinne 54
 Mrowka, Christian 39
 Mühl, Astrid 35
 Muller, Yves A. 50, 51, 52
 Müller, Anita 21, 52
 Müller, Annekathrin 56
 Müller, Dominik 26, 27
 Müller, Eva-Christina 44, 45, 60, 98
 Müller, Gerd 71, 105
 Müller, Gerda 130
 Müller, Jürgen J. 50, 52
 Müller, Thomas 36, 37, 142
 Müller, Uwe 50, 52

N

Nägler, Karl 134
 Neff, Klaus 25
 Neichel, Dajana 115
 Neubauer, Helga 60
 Neuhaus, Yvette 35
 Neumann, Claudia 109
 Nguyen-Hoay, Tam 106, 107
 Niss, Knut 66
 Nissen, Eberhard 97, 115
 Nitschke, Ute 105
 Nitz, Monika 23
 Nolte, Christiane 130
 Nörenberg, Ursel 137
 Nothnagel, Anita 30, 43
 Nürnberg, Gudrun 29, 43

O

Öczelik, Chemil 36, 37
 Ohl, Lars 25, 71
 Ohlemeyer, Carsten 130
 Otto, Albrecht 44, 45, 60, 98, 106

P

Pachale, Dietlind 29
 Pagel, Ines 85
 Pankow, Harald 43
 Pantelic, Liliana 21
 Papst, Marion 72
 Pecher, Gabriele 71
 Pei, Weidong 33, 35
 Peter, Heidrun 124
 Peter, Jens-Uwe 23
 Peters, Niels 103, 105
 Petretti, Tobias 90
 Pevzner, Veniamin 71
 Pezzutto, Antonio 80, 102, 106, 107,
 117, 120, 160
 Pfitzmaier, Birgit 85
 Pfrieder, Frank W. 128, 133, 134,
 142, 160
 Pidde, Heiko 31, 32, 89, 90
 Pietsch, Hubertus 66
 Plöhn, Sören-Peter 143, 160
 Pohl, Bärbel 112
 Pommeranz, Sascha 29
 Poppe, Brunhilde 43
 Poppe, Konstanze 30, 31, 32
 Posner, Steffen 71
 Poßling, Alexandra 124
 Preiß, Susanne 117
 Prokoph, Helmuth 31, 32

Q

Qin, Zhihai 107, 117
 Quass, Petra 97
 Quensel, Christina 88

R

Radau, Boris 98
 Räder, Charlotte 41
 Radtke, Susan 23
 Rahn, Hans-Peter 88
 Rappert, Angelika 130
 Rathjen, Fritz G. 128, 135, 137, 160
 Rechmann, Stefanie 25
 Reich, Jens 19, 35, 42, 43, 151, 160
 Reis, André 13, 19, 28, 29, 34, 160
 Reiterer, Philipp 71
 Reszka, Regina 15, 102, 110, 112, 151, 160
 Richter, Gerhild 77
 Richter, Günther 106, 107, 117
 Richter, Jana 15, 112
 Rickers, Anke 60, 103, 105
 Riechert, Freya 103, 105
 Ried, Christian 71, 97
 Riedel, Heidi 124
 Rieffel, Christine 143, 160
 Riege, Dorothea 83
 Riethmacher, Dieter 13, 36, 37
 Rietzke, Helga 100
 Rink, Sinaida 112
 Ristau, Otto 45
 Ritschel, Silke 105
 Roefzaad, Claudia 90
 Rohde, Klaus 30, 43
 Rohs, Remo 56
 Rösch, Marion 117
 Roske, Yvette 50, 52
 Rossum, Denise van 112
 Röttschke, Olaf 13, 118, 160
 Royer, Hans-Dieter 73, 74, 105, 160
 Rucker, Karin 30, 32
 Rudolph, Cathrin 37
 Rudolph, Sabine 81
 Rüschenndorf, Franz 29, 43
 Ruiz, Patricia 77

S

Saar, Kathrin 29
 Sacharjat, Michael 124
 Sachs, Martin 76, 77
 Sawitzki, Bianca 29
 Schäfer, Michael 137
 Schäfer, Olaf 60, 97
 Schaenke, Michaela 130
 Schaeper, Ute 76, 77
 Scharschmidt, Erika 68
 Scharte, Gudrun 31, 32
 Schaup, Annette 58
 Schedl, Andreas 18, 38, 39, 160
 Scheel, Sabine 21
 Scheer, Helga 122
 Scheidereit, Claus 63, 67, 68, 74, 103, 105, 142, 160
 Schenk, Harry 149, 160
 Schenk, Jörg 31, 32
 Scherneck, Siegfried 19, 30, 32, 89, 90, 160
 Schildt, Jürgen 122
 Schipke, Carola 130
 Schirrmann, Thomas 71
 Schlag, Peter M. 8, 15, 19, 31, 32, 42, 78, 79, 80, 89, 90, 123, 124, 142, 151, 160
 Schlegel, Wolfgang 83
 Schlisio, S. 123
 Schmid, A. 73, 74
 Schmidt, Folke 27
 Schmidt, Hannes 137
 Schmidt, Holle 115
 Schmidt, Mathilde 27
 Schmidt, Susanne 29
 Schmidt, Sabine 39
 Schmidt, Steffen 43
 Schmidt, Willy 43
 Schmidt-Ullrich, Ruth 68, 142
 Schmitz, Christian 41
 Schneider, Frank 90
 Schneider-Mergener, Jens 57, 58
 Schnitzer-Ungefug, Jutta 160
 Schnitzler, Mark 107
 Schüler, Thomas 117
 Schultz, Jörg 43
 Schulz, Hannelore 41
 Schulz, Wolfgang 56
 Schulze, Bettina 90
 Schumacher, Karin 90
 Schumacher, Stefan 13, 137
 Schumann, Margitta 60
 Schunck, Wolf-Hagen 26, 93, 94, 100, 160
 Schuster, Herbert 19, 25, 33, 35, 160
 Schuster, Stefan 43
 Schütz, Gunnar 77
 Schwabe, Holger 89, 90
 Schwartz, Arnfried 31, 32
 Schwarz, Dieter 94
 Schwarz, Monika 29, 106, 107
 Schwarz-Romond, Thomas 78, 79
 Schwenkenbecher, Jan 104, 105
 Seeger, Michaela 29
 Seehrich, Hans-Joachim 160
 Seelow, Dominik 29
 Seemann, Stefanie 112

Seibert, Patric 103, 105
 Seidensticker, Martin 77
 Seitz, Susanne 30, 31, 32
 Sendtner-Voelderndorff, Luzi 137
 Shakeri-Garakani, Ansiah 124
 Shan, Ying 73, 74
 Sharma, Kamal 137
 Shin, Jung-Bum 132
 Sieber, Martin 37
 Siemer, Ansgar 47
 Silva Junior, José-Antonio 23
 Siuniaev, Chamil 43
 Sirtes, Daniel 130
 Sklenar, Gisela 51, 52
 Sklenar, Heinz 19, 55, 56, 160
 Skorna, Madeleine 29
 Sochnikova, Natalia 75, 77
 Sommer, Thomas 13, 91, 92, 99, 160
 Sonnenberg-Riethmacher, Eva 36, 37
 Spillmann, Freia 29
 Spingies, Christine 29
 Sporbert, Anje 88
 Stade, Katrin 92
 Stahn, Renate 80, 81, 121
 Stein, Ulrike 89, 90
 Steinert, Fabian 29
 Steinhusen, Ulrike 105
 Sternke, Anne 94
 Stoffels, Mandy 27
 Stolley, Peter 143, 160
 Strauss, Holger 112
 Strauss, Michael 13, 14, 68, 124, 150, 160
 Strehle, Michael 36, 37
 Stucky, Cheryl L. 131, 132
 Stulich, Marianne 122
 Sturm, Isrid 103, 105, 107
 Stüwe, Eva 126
 Sugimura, Keiichi 23
 Sümnich, Ingeborg 30, 32
 Sydow, Hanna 85
 Synowitz, Michael 130
 Szangolies, Inka 29
 Szelestei, Tamas 35

T

Tank, Jens 26, 27
 Tegethoff, Sebastian 68
 Teppke, Anne-Dorothee 121
 Theile, Michael 32
 Theuring, Sebastian 105
 Thiele, Verena 43
 Thierfelder, Ludwig 160
 Tian, Xiao-Li 21
 Timmermann, Bernd 25
 Tobias, Doerks 43
 Toka, Hakan 33, 35
 Tönjes, Michael 77
 Trautner, Thomas A. 160
 Trotier, Fabienne 29
 Tschapek, Alexej 43
 Tünnemann, Gisela 52

U

Uckert, Wolfgang 117, 142
Uhlmann, Regina 35
Ulrich, Oliver 31, 32, 89, 90
Urban, Jörg 92

V

Varon-Mateeva, Raymonda 29
Vetter, Donathe 83
Vidal, Valerie 39
Vogel, Frank 94, 99, 100
Vogel, Margit 99, 100
Vogel, Regina 75, 76, 77
Volkwein, Corinna 92

W

Wallukat, Gerd 15, 41, 96, 102, 114,
115, 142, 150, 160
Walter, Jan 92
Walther, Diego 23, 41
Walther, Thomas 23, 85
Walther, Ingrid 76, 77
Walther, Wolfgang 89, 90, 121
Wedekind, Brigitta 37
Wegener, Monika 115
Wegner, Daniela 112
Weinert, Ingeborg 74
Welfle, Heinz 19, 57, 58, 160
Welfle, Karin 58
Weller, Andrea 29
Wellner, Maren 95, 97
Wenzel, Katrin 25, 80
Werner, Erik 50, 52
Werner, Sabine 31, 32
Wessel, Ralf 99, 100
Westen, Christel 117, 142
Westermann, Jörg 106, 107, 120
Westermann, Peter 98, 160
Wetzel, Katarina 85
Wetzel, Ulrike 85, 100
Wieske, Martin 99, 100
Willimsky, Gerald 117
Willnow, Thomas E. 15, 18, 26, 40,
41, 151
Winbeck, Georgia 75, 77
Winkler, Liselotte 23
Wirtz, Ralph 78, 79
Witting, Anke 130
Wittmann-Liebold, Brigitte 19, 59,
60, 103, 106, 160
Wittstruck, Angelika 92
Wiznerowicz, Irmgard 77
Woischwill, Christiane 73, 74
Woldeyesus, Mas 36, 37
Wolf, Edelgard 43
Wolff, Gerhard 73, 74, 105
Wuchter, Christian 104, 105
Wunderlich, Heidrun 81
Würtele, Martin 77, 78, 79

Y

Yuan, Yanping 43, 49

Z

Zacharias, Martin 56
Zacharias, Ute 137
Zacharzowski, Udo 85
Zastrow, Inga 35, 43
Zechner, Dietmar 77
Zeidler, Helga 30, 32
Zeisig, Reiner 121
Zenke, Martin 100, 102, 106, 119,
120, 160
Zenker, Jana 39
Zhaoqiang, Cui 21
Zhou, Lepu 79
Ziegler, Ulrike 39
Zimdahl, Heike 21
Zinke, Silke 35
Zirwer, Dietrich 47
Zummach, Ramona 94
Zuske, Alexandra 90

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