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NGFN
National Genome
Research Network

SCIENCE INSIDE

THE NATIONAL GENOME RESEARCH NETWORK

FOREWORD

"We used to think our fate was in the stars. Now we know, in large measure, our fate is in our genes." With his often quoted comment, the biologist and Nobel Prize laureate James Watson describes what we now know about the significance of genes for health. Genetic factors play a crucial role in the development of most – if not all – human diseases. They can cause disease onset, increase disease risk, influence the course of a disease and modify the effectiveness of drugs.

Like hardly any other scientific field, human genome research has changed our understanding of the causes of human diseases and has rightly taken its place in medical research. Cutting-edge technologies and systematic research approaches have led to a dynamic increase in knowledge. Findings from human genome research have become increasingly significant for medical practice. Already today, the diagnosis, therapy and prevention of many diseases are based on insights gained about the molecular causes of disease. Individualized medicine seems to be within our reach.

Early on in the research to understand the genetic causes of disease, it became evident that one scientific discipline alone soon comes up against its limits. Intensive interdisciplinary cooperation is necessary between genome researchers, clinicians, statisticians, engineers and other specialists, especially because many diseases are elicited by a complex interaction of various genes and by an interaction of genes with the environment. In Germany, the National Genome Research Network (NGFN) is advancing interdisciplinary cooperation in a scientific network that is unique in the world. Scientists here have joined together to utilize the new chances of interdisciplinary research and to create a visible added value for society. The approaches pursued for this are quite diverse. They span clinical research, systematic approaches and individual Explorative Projects, whose significance will first become apparent in the future.

Since 2001 the German Federal Ministry for Education and Research (BMBF) has been funding the NGFN to promote the elucidation of the genetic causes of common diseases. So far, this research work has been very successful. In the past four years the disease genes for allergies, chronic inflammatory bowel diseases, alcohol addiction, epilepsy and Parkinson's disease have been discovered. The development of DNA chips which can help detect disease-relevant gene mutations in kidney and breast cancer, leukemia or congenital heart diseases has attracted international attention.

The NGFN has given disease-oriented human genome research a new dimension in Germany, and with this brochure we would like to share this with you. Reports about selected projects will give you insight into the work of each individual research focus. Look over our shoulders! If we have awakened your interest, an enclosed CD-ROM with descriptions of all NGFN research projects will provide you with more information.

We wish you fascinating reading!

Professor Annemarie Poustka
- **Spokesperson of the Project Committee of the NGFN**
- **Director of the Department of Molecular Genome Analysis at the German Cancer Research Center in Heidelberg**

Professor Stefan Schreiber
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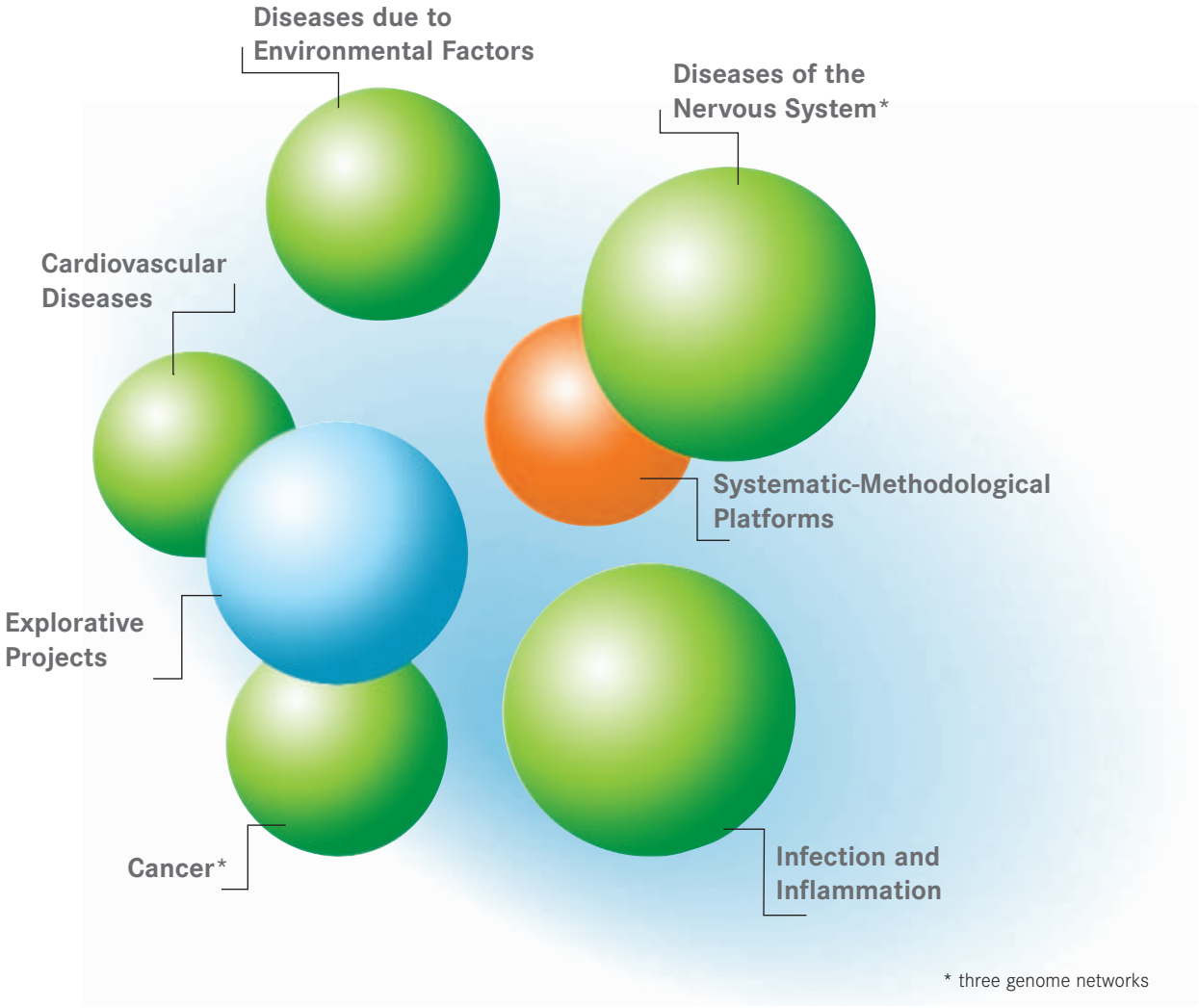
THE NATIONAL GENOME RESEARCH NETWORK

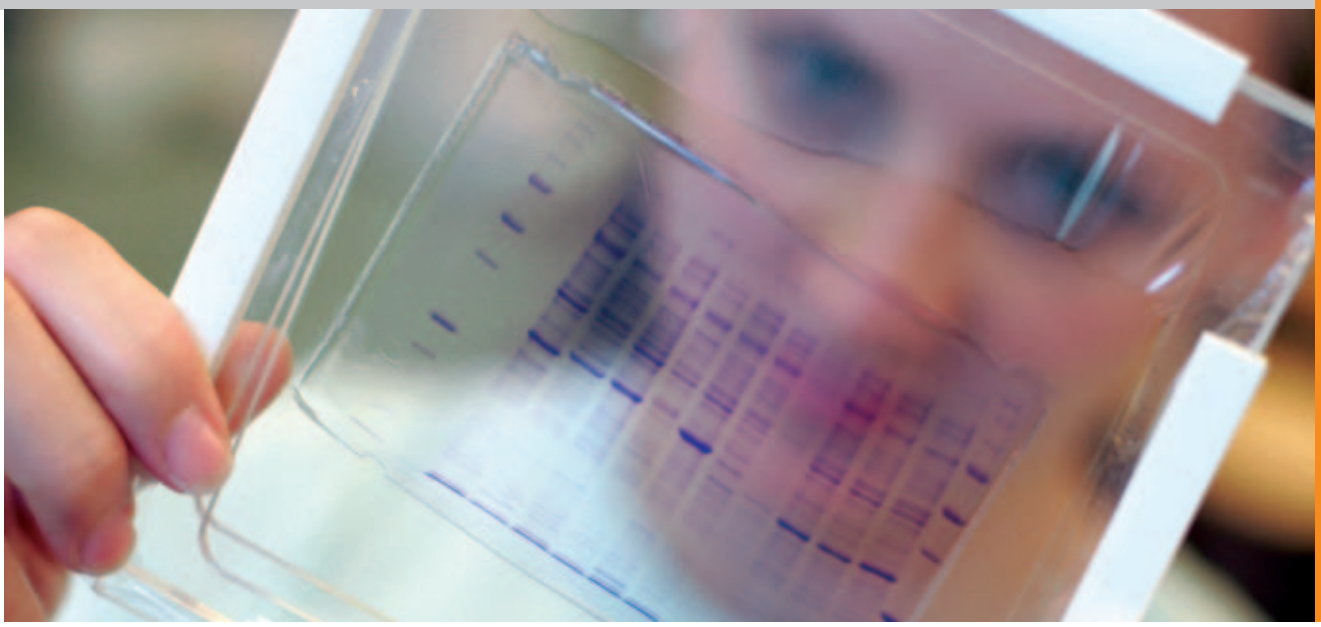


The research activity of the National Genome Research Network (NGFN) focuses on investigating genetic causes of common diseases. To accomplish this, a unique organizational structure has been created so that leading experts from both systematic genome research and clinical research can collaborate closely. Together, they seek to understand the complex control mechanisms of the human body at the DNA, RNA and protein level in order to find keys to new treatments for currently incurable diseases.

The NGFN is continuing the successful work of the German Human Genome Project (DHGP), which between 1995 and 2004 conducted basic genetic analyses to decode the human genome and continued this research with functional analyses. Thus far, the achievements of the NGFN scientists have been impressive: Already in the initial grant period from 2001 to 2004, the scientists' research efforts led to significant results. In 2003 the work of the NGFN was evaluated by an international panel of experts. Based on the findings of this evaluation, the German Federal Ministry for Education and Research (BMBF) has awarded a second grant to the NGFN extending through 2007.

DISEASE-ORIENTED AND SYSTEMATIC GENOME RESEARCH

Working within nine Disease-oriented Genome Networks, NGFN scientists are investigating the processes underlying diseases of the nervous system, cardiovascular diseases and cancer. They are also studying diseases caused by infection, inflammation or environmental factors. NGFN's patient-

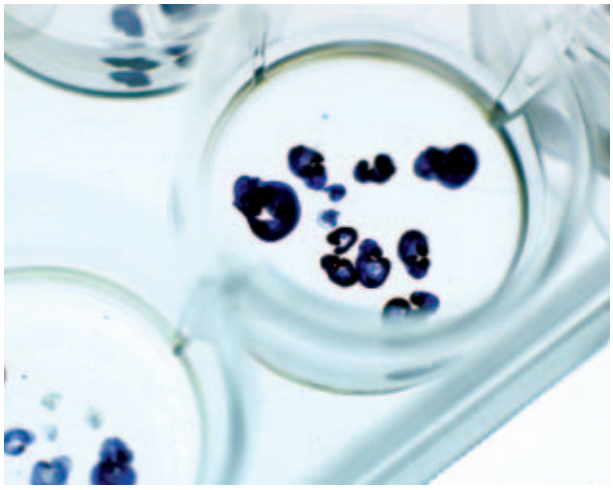


Protein separation by gel electrophoresis

oriented research work goes hand in hand with research involving systematic genome, transcriptome and proteome analysis. The know-how for such large-scale research initiatives is provided by twelve Systematic-Methodological Platforms (SMP). Highly specialized experts work in these SMP, utilizing and continually developing powerful technologies for modern high-throughput research. In addition, they provide all NGFN scientists with efficient data processing systems. Since 2004, nineteen Explorative Projects (EP) have been created in which NGFN scientists can test and implement their innovative research ideas. The aim of these EP is to generate new technologies and to open up additional fields of application for disease-related human genome research.

ESTABLISHING STANDARDS AND APPLYING RESULTS

A comprehensive quality management system enables all NGFN scientists to comply with the same quality standards, which are in accordance with international guidelines.



Isolated mouse embryos

The QM system ensures the consistent high quality of all materials and results produced by the NGFN as well as the optimal exchange of data within the network. The NGFN has also established a central coordination office for technology transfer. Its task is to identify research results generated in the NGFN that might prove economically valuable and to speedily facilitate their commercialization by making them available to industry. This directly benefits patients by increasing their opportunities to access the new therapies and diagnostic methods developed by the NGFN.

ADVISING, COORDINATING AND ORGANIZING

The research focus and the scientific strategy of the NGFN are primarily determined by the steering board, which functions as an independent, external body. It guides and supervises program implementation, thus supporting the NGFN's development. Membership on the board is an honorary position, and the eight members are prominent representatives of the academic and industrial research communities. Internally, the NGFN is managed by a project committee, which is comprised of representatives of the Systematic-Methodological Platforms, the Disease-oriented Genome Networks as well as one member representing the Explorative Projects. The members of the Project Committee monitor the course of the scientific projects and coordinate research and public relations activities. The Project Management provides operational support to the Project Committee. It regularly reports on the current situation and the development of the entire NGFN and also organizes scientific conferences. Through its public relations work, the project management seeks to promote public acceptance of medical genome research.

Nikolas von Bubnoff

GETTING THE EDGE ON CANCER – PREDICTING RESISTANCE PROFILES

When combating cancer diseases, doctors sometimes feel they are fighting a clever opponent and not a disease: Just when a medication is found that is successful, the cancer cells fight back with mutations, making it ineffective. The same is true for chronic myeloid leukemia (CML). Since 2000, Professor Justus Duyster and his team in the Network "Combating Cancer through Integrated Functional Genomic Research" of the NGFN have been focusing specifically on preventing these drug-resistant relapses. In CML a reciprocal translocation between chromosome 9 and the long arm of chromosome 22 leads to the development of a dangerous fusion protein: tyrosine kinase Bcr-Abl. It is present in nearly all leukemia cells. This kinase permanently activates the Ras-Raf-MEK kinase signaling pathway, thus enabling the cells to proliferate in an uncontrolled way. Presumably, CML begins in the bone marrow in a single pluripotent stem cell, a myeloblast, from which granulocytes arise. In patients with CML, an abnormally high number of granulocytes and immature precursors of these cells are present both in the bone marrow and in the blood. In the first years the disease responds well to treatment, in fact many patients do not exhibit any symptoms. This so-called chronic phase can last for up to six years. But over time the leukemia becomes more aggressive. In the subsequent advanced stages, a transitional phase with an accelerated growth of the leukemic clone is distinct from the final blast phase, in which an excessive number of immature blood cells (blasts) are present in the bone marrow and in the blood. The blast phase ends fatally for all patients after three to six months.

CLEAR POINT OF ATTACK

At the end of the 1980s scientists already had a molecular understanding of CML, and they envisioned a very promising new therapy approach: inhibiting tyrosine kinase Bcr-Abl. In 2001 the kinase inhibitor Imatinib Mesylate finally came on the market. It targets the ATP-binding region of the enzyme. Thus, ATP cannot bind and Bcr-Abl is inactive. The cancer cells no longer divide and finally die due to apoptosis. The objective of the therapy, the disappearance of all cells harboring the chromosomal translocation (cytogenetic remission), can be attained with Imatinib in over 90 percent of the CML patients who are in the chronic phase. "That was a tremendous success, because with previous treatment methods only a very small group of patients could be helped," Justus Duyster recalls. But unfortunately there is a down side as well. In the accelerated phase of CML relapses occur already after six to nine months. The cancer cells become resistant to Imatinib and proliferate as they did before. This has spurred several research teams to begin searching for the molecular causes of this resistance. In addition to amplifications of the *Bcr-Abl* gene, overexpression of the Bcr-Abl kinase and elevated expression of the multidrug resistance protein MDR1, point mutations were found in the kinase domain of the protein. Dr. Nikolas von Bubnoff, group leader in Justus Duyster's lab, studied seven patients who had a relapse while being treated with Imatinib: "We found five distinct point mutations that either affect the ATP-binding domain or the activation domain," says Nikolas von Bubnoff. All five mutations change the structure of the kinase in such a way that Imatinib can no longer

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bind. However, the ATP binding remains unimpaired, so that the kinase is active. "In more than 70 percent of all CML patients with a relapse studied up till now, such point mutations were discovered. They are thus the primary cause for the Imatinib resistance," he adds, summarizing the findings of the different research groups.

A GOOD ALTERNATIVE

In the face of the occurring Imatinib resistance the scientists began to look for alternative kinase inhibitors. They pursued research utilizing the chemical class of pyrido-pyrimidines (PD). Crystal structure analyses showed that pyrido-pyrimidines also bind to the ATP-binding site of the kinase, but in a different way and apparently better than Imatinib. The Duyster research group studied 13 different pyrido-pyrimidines. All tested substances inhibited Bcr-Abl and did so with considerably lower concentrations than Imatinib. And most important of all, they were also able to inactivate clinically relevant Imatinib-resistant forms of the kinase. Conversely, pyrido-pyrimidines evoke in part mutations that can be inhibited with Imatinib. In future, the physicians therefore plan to combine different inhibitors in the treatment of CML from the very beginning or to administer them sequentially. Two of the alternative kinase inhibitors are already in clinical phase I and II trials. "The first results from a study with 107 patients are very promising. Perhaps alternative kinase inhibitors will be available within two or three years for CML therapy," Justus Duyster hopes.

PREDICTING RESISTANCE PROFILES

For the new therapy schemes, knowing which substance triggers which mutation prior to use on patients would be of great value. Lengthy, complicated clinical studies would then become unnecessary. Based on these considerations, Duyster's team has developed a cell-based method with which resistance mutations to kinase inhibitors can be predicted. "Of course, for this it was crucial that the occurring mutations in this cell culture system are also clinically relevant," Justus Duyster explains. To accomplish this, the Munich team produced a cell line that expresses Bcr-Abl and can only survive when this kinase is constitutively active. First, they studied the effect of administering Imatinib to the cells in the concentrations which CML patients receive. "Initially, the cell culture dishes remained empty," says Jana Sänger, technical assistant in the Duyster lab. Then after six or seven weeks the first resistant colonies grew. Sequencing of the Bcr-Abl domain of these clones revealed mutations that are also present in CML patients. Currently, by means of its cell culture system, the Munich group is specifically investigating such substances that are used in therapy studies. For instance, tests with the pyridopyrimidine PD166326 showed that cells treated with this substance were less often resistant than with Imatinib.

Moreover, quite different Bcr-Abl mutations appeared. In part, these could be suppressed quite easily by increasing the PD166326 concentration. Three more mutations could be suppressed with Imatinib. "The advantage of the cell culture system is that it is possible to test in advance which mutations occur with which substances and which inhibitor can be used as an alternative," Nikolas von Bubnoff explains. "Thus, therapy strategies can be derived directly from our system." The Munich scientists are also applying their method to other forms of cancer which have similar mechanisms for the formation and development of resistance. He adds, "I am firmly convinced that targeted treatment strategies can be directly derived from our cell culture findings, which will improve the chances of healing cancer in the future."

Petra Seipel (Duyster group)

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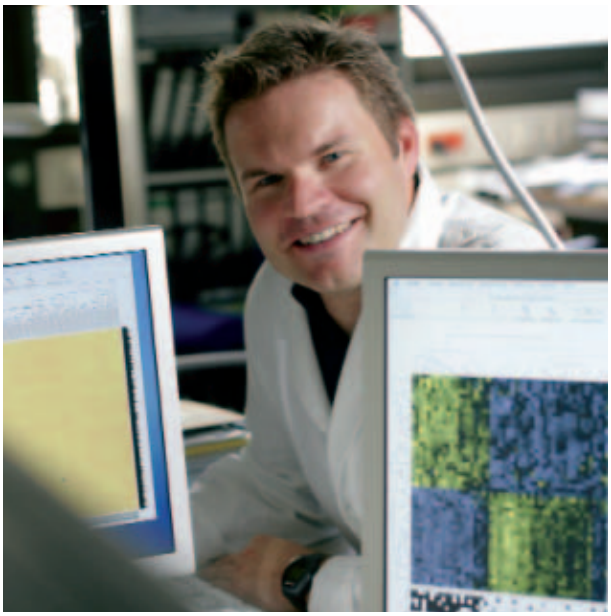
CANCER IN CHILDREN – GENETIC ANALYSES ALLOW PERSONALIZED THERAPY

Terrible. Shocking. Unfair. When children get cancer, feelings of sadness are overwhelming. The emotional impact of each child's illness is immense. We have an acute need to find an explanation: Why do the young and innocent have to suffer so much? But perhaps cancer specifically in children holds the key to this still baffling disease. "When cancer develops so early in life, the suspicion is warranted that genetic factors are mainly responsible for the cells' rampant growth. Negative environmental influences or an unhealthy lifestyle have not had enough time to affect the young organism," says Professor Manfred Schwab of the NGFN. "We believe that by investigating tumors in children, we will be able to better understand the genetic causes of cancer overall." Manfred Schwab and Professor Angelika Eggert of the University Hospital Essen have gathered together a team of scientists intent on combating neuroblastoma, the most frequent solid tumor in children. Of 100,000 girls and boys, between one and three will suffer from this insidious malignancy of the sympathetic nervous system. The NGFN researchers are working together in the Cancer Network "Systems Biology of Embryonal Tumors – Neuroblastoma as a Model".



Frank Westermann

SOMETIMES THE CANCER GOES AWAY BY ITSELF

All in all, neuroblastoma has a bad prognosis. Only about every second child survives the disease. However, the course of the disease varies extremely. In about ten percent of the cases spontaneous healing is observed. That means the tumor goes away by itself – just as mysteriously as it appears. Scientists are convinced that the rather benign and the extremely malignant tumors are genetically distinct. "If we could say, based on gene analysis, whether a sick child is suffering from an aggressive neuroblastoma or whether a spontaneous remission can be anticipated, it would be easier to choose the appropriate therapy from the very beginning," explains Manfred Schwab. "We would know which children definitely need an intensive treatment despite the serious side effects, and whom we could spare this therapy." Precedence for this approach is the *MYCN* gene copy number, which defines a subgroup of high-risk patients and is used worldwide as a robust stratification marker in neuroblastoma. However, a considerable number of aggressive neuroblastomas lack amplified *MYCN*, indicating that other unfavorable molecular events are involved in neuroblastoma tumor progression. In addition, the elucidation of the genetic causes of neuroblastoma should provide approaches for new therapies.

A SMALL PIECE ON CHROMOSOME 1 IS MISSING

In the research on neuroblastoma in the Cancer Network, a number of genetic factors have already been identified which allow assertions to be made about the aggressiveness of the tumor and thus about the prognosis of the young patients. One of these genetic factors is called *CAMTA1*, which scientists of the German Cancer Research Center (DKFZ) in Heidelberg discovered. They happened upon *CAMTA1* while investigating which genes are located on a small fragment of the short arm of chromosome 1. In previous studies they found that this fragment is often missing in neuroblastoma. "Thus, the obvious conclusion was that the missing fragment must contain genes that have something to do with the disease," explains Dr. Kai-Oliver Henrich, who together with Dr. Frank Westermann co-supervised this project. Using cDNA microarrays and real time RT PCR, Kai-Oliver Henrich and his colleagues correlated the expression of the gene to the different tumor characteristics. Result: if *CAMTA1* is only expressed to a small extent, the neuroblastoma appears to be particularly aggressive. "We think that *CAMTA1* can contribute to making the prognoses more accurate than they have been previously," he concludes. "Next, we want to clarify what exact function the gene has and how it is regulated. By this means we will be able to understand the biology of the neuroblastoma a little bit better and hopefully develop new therapies." Until now, it was only known that *CAMTA1* as a transcription factor might participate in the regulation of the cell cycle and also that it is expressed to a diminished degree even in other kinds of cancer, e.g. in certain brain tumors and in a form of skin cancer.

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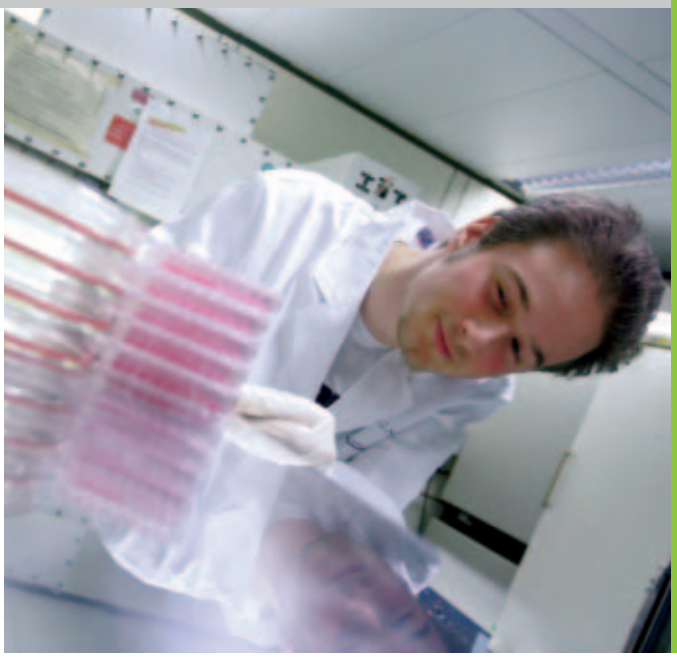

Steffen Bannert (Schwab group)

GENE EXPRESSION-BASED TUMOR CLASSIFICATION

A joint project within the Neuroblastoma Cancer Network to predict the prognosis of neuroblastoma by using gene expression analysis is being pursued by Dr. Matthias Fischer (University of Cologne) and Dr. Frank Westermann (DKFZ). Their hypothesis: The more genetic factors that are known through which neuroblastomas can be characterized, the more exactly the aggressiveness of the disease can be determined by using them. In collaboration with the partners within the Neuroblastoma Cancer Network the scientists at the DKFZ and the University of Cologne first devised a microarray with about 10,000 genes which are suspected of being somehow related to neuroblastoma. Matthias Fischer and his team have demonstrated the validity of this hypothesis. Based on this array, they prepared gene expression profiles of the very malignant neuroblastomas on the one hand and of relatively benign tumors on the other hand. Depending on the aggressiveness of the tumor, the profiles showed distinct differences. These differences enabled the identification of 144 genes which explicitly influence the prognosis of the disease. The expression pattern of the 144 genes was then investigated in 83 patients with a known prognosis, who suffered from neuroblastomas of varying aggressiveness. There it was shown that the gene expression profiles mirrored the prognosis of the disease very well – better than the usual classification systems used until now, which are oriented on the age of the patients, the tumor stage and histological characteristics. Dr. André Oberthuer, a member of Matthias Fischer's team, explains the next steps: "To ultimately clarify whether our system allows reliable statements about the further course of the disease, we now want to apply it prognostically. This means that we want to investigate the tumor tissue right at the beginning of the disease and then see whether our predictions come true. Until now we have only had to deal with tumors whose behavior was already known." If these new studies also have positive results, gene expression analysis could soon be routine for tumor classification. Matthias Fischer: "Then we can treat the affected children in a much more targeted way."

Malgorzala Sawinska (Schwab group)

The project of Professor Angelika Eggert's team at the University of Essen is at a similar stage. Using a commercially available biochip, she and her colleagues have identified 39 genes which likewise seem to characterize the malignancy of the neuroblastoma. By means of gene expression they could track the course of the disease in 68 of the afflicted children with an accuracy of 80 percent. "Interestingly, however, there was hardly any overlap between our results and the results of our colleagues from Cologne. This means that only a few genes were considered to be prognostically relevant by both research groups. Angelika Eggert is therefore awaiting with great interest the findings of the planned prospective studies from Cologne, and she is planning to supplement the gene expression analyses with studies on the protein level.

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WHICH GENES CONTROL BRAIN TUMORS?

On average, a patient has less than twelve months left to live when he is diagnosed with glioblastoma – despite intensive radiation – and chemotherapy. Glioblastomas are the most common and malignant form of brain tumors with one of the lowest survival rates of all types of cancer. "At present, we do not yet have any curative therapy for glioblastoma," says Professor Otmar D. Wiestler. He coordinates the Disease-oriented Genome Network Brain Tumor Network (BTN) in the NGFN. "That is why we are focusing on glioblastomas in our research network." Glioblastomas originate like several other brain tumors from so-called glial cells. These support the nerve cells and ensure that an optimal milieu is maintained for them.

Björn Tews, Meinhard Hahn and Sebastian Barbus

MISSING PIECES OF CHROMOSOME

In the Brain Tumor Network the research groups of the university hospitals of Berlin, Bonn, Düsseldorf, and Tübingen have joined together with colleagues of the German Cancer Research Center (DKFZ) in Heidelberg. The BTN wants to elucidate what happens on the molecular level when brain tumors develop. The aim is to more accurately prognosticate tumors, thus enabling the development of new therapeutic approaches. For glioblastoma the scientists have already detected a number of chromosomal and genetic changes – in particular, potential tumor suppressor genes – in the mutated cells. Such genes regulate the cell cycle and prevent the development of cells that proliferate in an uncontrolled fashion. If tumor suppressor genes go missing or if they are defective, tumors can easily develop. A conspicuous observation led researchers to conclude that tumor suppressor genes are possibly lacking in glioblastoma. The same segments of chromosome 10 and/or chromosome 22 were always absent in the tumor cells of most of the patients.

WHICH GENE PRODUCTS ARE AFFECTED?

BTN researchers led by Professor Guido Reifenberger and Dr. Marietta Wolter of the University of Düsseldorf have identified four genes which are situated in the region in question of chromosome 10 and which are expressed to a lesser degree in glioblastoma. Professor Andreas von Deimling and Dr. Christian Hartmann of the Berlin Charité have similarly succeeded with astrocytoma, a frequent precursor tumor of glioblastoma. Both of them made their discoveries on the missing piece of chromosome 22. The potential tumor suppressor genes, which the scientists in Düsseldorf and Berlin discovered, are now being investigated more closely at the DKFZ in Heidelberg. "We want to elucidate the disease-specific regulation processes and the function of these genes," says Dr. Meinhard Hahn, project leader at the DKFZ. The Heidelberg BTN research groups are specialized in molecular genetic high-throughput analyses. Among other achievements, Meinhard Hahn and his colleagues have devised a special microarray with which they can create comprehensive RNA expression profiles. The research group of his colleague Dr. Bernhard Radlwimmer is analyzing mutations of the genomic DNA using array-based comparative genomic hybridization (CGH). "It is especially interesting to correlate the

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changes on the level of DNA and RNA with each other," Meinhard Hahn says. "To accomplish this, we are developing special data analysis methods and computer programs." The basis of the genetic analyses is the so-called Glioma Core Collection. This is a collection of 100 very well documented tumor samples representing the different stages and forms of glioma. The tissue database was built up in a collaboration of the Düsseldorf and Berlin working groups.

fore is expressed more abundantly." The basis for this discovery was a tissue database with samples of 200 medulloblastomas. They originate from patients whose medical history is known and well documented. "In the future, by means of CDK6, the prognosis of the patients can be assessed more reliably and the therapy adapted accordingly," he hopes. "Moreover, the protein is well suited for immunological detection in routine diagnostics."

Ute Schmidt (Lichter group)

NEW PROGNOSTIC MARKER FOR BRAIN TUMORS IN CHILDREN

Another tumor the BTN is studying is medulloblastoma. It is one of the most common malignant brain tumors in children. For about half of the patients this tumor is fatal. The BTN research group of Bernhard Radlwimmer and Frank Mendrzyk at the DKFZ has now identified a gene that could possibly enable prognoses for the afflicted girls and boys. According to their research findings, an overproduction of the protein CDK6 seems to accompany an unfavorable course of the disease. The gene for CDK6 is situated on chromosome 7 and apparently has a key function for the growth and differentiation of medulloblastoma cells. "CDK6 seems to allow more accurate prognoses for patients with medulloblastomas than already known markers," says Bernhard Radlwimmer. "The cause of the overproduction of CDK6 is that the responsible gene occurs too frequently in the tumor cells and there-

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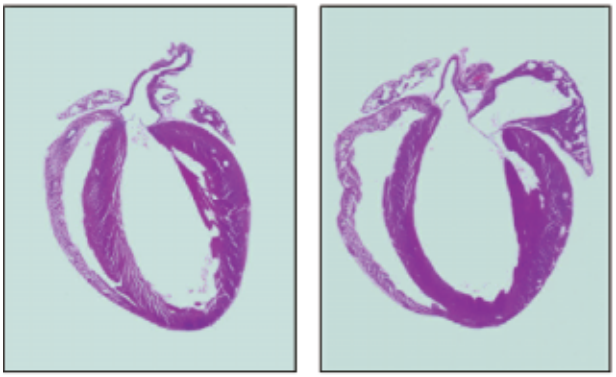
IMPACT OF GENES ON THE MOTOR OF OUR LIVES

Today it is common knowledge that heart attack patients require immediate medical attention and thorough aftercare. But what about their relatives? We should get into the habit of scrutinizing not only the patient, but also the patient's family," says Dr. Christian Hengstenberg, cardiologist at the University Clinic of Regensburg. He and his colleagues have been researching the genetic basis of heart diseases for a number of years – and with success. Only recently, the scientists of the Disease-oriented Genome Network Cardiovascular Diseases were able to show that genetic factors determine not only the onset of coronary heart disease (CHD), but the course and severity of the disease as well. To a great extent, the presence of calcium deposits and the distribution of vascular stenoses are determined by genes. In particular, stenoses of the aorta and the proximal segments of the coronary arteries are hereditary. "If a patient with coronary heart disease shows such an affliction pattern, we should perform a medical check-up on the patient's relatives as to their risk of developing CHD. Simple screening tests are sufficient to enable us to react soon enough," Christian Hengstenberg explains. And that can save lives, because stenoses of the main coronary vessels and the proximal segments of the coronary arteries are especially dangerous. They often lead to heart rhythm disturbances and myocardial infarction – complications that are fatal for many afflicted people.

FAMILY-BASED PREVENTION

Under the direction of Professor Heribert Schunkert from the University Clinic of Regensburg, Christian Hengstenberg and his colleagues studied the cardiac catheter films of 882 siblings from 401 families. The selection criteria for the families were that the index patient had suffered a heart attack prior to his or her 60th birthday and that at least one sibling suffered from a severe coronary heart disease. "As far as we currently know, our project is the first to distinguish between the individual forms of coronary heart disease and to assign the significance of hereditary factors to the respective disease picture (clinical phenotype)," Heribert Schunkert says. The results are especially important so that preventive care can be initiated for relatives of heart attack patients who have no symptoms yet. Christian Hengstenberg refers to this as "family-based prevention". Next, the scientists want to identify the genes responsible for the different forms of coronary heart disease.

Preparation for DNA gel electrophoresis



Calsarcin-deficient mouse heart (right), displaying dilated cardiomyopathy in response to aortic banding. A wild-type heart (left) subjected to banding served as control.

ONGOING HEART RESEARCH

The project of Schunkert, Hengstenberg and colleagues is only one of many in the genome network Cardiovascular Diseases of the NGFN. Since 2001 investigations have been ongoing there to determine the genetic causes of coronary heart disease and heart attacks. Other groups are successfully researching hypertension, cardiac insufficiency and cardiomyopathy. For instance, researchers on the team of the Heidelberg scientist Dr. Wolfgang Rottbauer discovered one of the genetic causes for cardiac insufficiency. Their study on zebrafish showed that mutations in the gene *dead-beat* reduced the pumping capacity of the heart. Dr. Norbert Frey and his colleagues at the Medical University Clinic Heidelberg moved a decisive step forward in the field of cardiomyopathy: They found a genetic mechanism which could play a decisive role in the onset and development of cardiac insufficiency. For this achievement they were awarded the Franz Maximilian Groedel Research Prize of the German Cardiological Society. In the animal model the researchers detected calsarcin-1, a protein that is primarily present in the heart muscle cells and is coded by the gene *Myoz2*.

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Norbert Frey

ADDITIONAL STRESS TRIGGERS DISEASE

To see what function calsarcin-1 has, the scientists purposely switched it off in mice. "Our mice mutants showed no disease symptoms whatsoever and had a normal life expectancy up to the point in time we exposed them to an additional stress factor," Norbert Frey explains. Then, for instance, the increase in blood pressure had fatal consequences: The animals developed a pathological enlargement of the heart (dilated cardiomyopathy). Calsarcin-1 does not seem to play any role for the pre- and post-natal development of the heart. But for mice without calsarcin-1 a specific gene program was active from birth on which could be responsible for the fact that the animals reacted to an increase in blood pressure with an exaggerated, pathological enlargement of the heart.

The results indicate that calsarcin-1 plays a key role in the function and modulation of heart muscle cells. As response to biomechanical stress, e.g. elevation in blood pressure, it seems to trigger a specific biological reaction that leads to heart enlargement. The enzyme is accordingly an important component of the molecular machinery of the heart muscle cells. It enables the heart to adjust to increased strain.

HOPE FOR NEW THERAPY OPTIONS

"Calsarcin-1 is also present in human hearts. We therefore surmise that it plays an important role in human heart muscle diseases," Norbert Frey says. He and his colleagues are now investigating whether a gene mutation exists in patients with heart muscle diseases which has a functionless calsarcin-1 as consequence. He adds, "Possibly calsarcin-1 could even be the starting point for a new treatment option to improve the adjustment of the heart to high blood pressure."

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Armin Heils

RESEARCHERS AS CHANNEL WORKERS – SEARCHING FOR THE GENETIC CAUSES OF EPILEPSY

Hardly any other disease has been given as many different names in the course of history as epilepsy. "Holy disease", "lunacy", "demonic disease" or even the "scourge of Christ" are only a few examples. Today we know much more about the actual causes of epilepsy. Nevertheless, we can still describe it as a disease with a thousand names. In fact, the term epilepsy covers a great number of different disease forms. What they all have in common is the repeated occurrence of seizures. In the Network "Systematic Gene Identification and Functional Analyses in Common CNS Disorders" of the NGFN several groups are studying epilepsy. In their search for the genetic causes of this seizure affliction they have already achieved some striking successes, as for example in Bonn, where the research group of Dr. Armin Heils is concerned with idiopathic generalized epilepsies (IGE).

MALFUNCTIONING CHLORIDE CHANNELS

Idiopathic epilepsies comprise about 40 percent of all epilepsies. In this form of the disease no clear cause in the brain can be found that triggers the seizures. Although ten genes have already been found that cause very rare forms of idiopathic epilepsies, the molecular background for the frequent subforms of IGE has not yet been elucidated. In a study investigating the complete genome of 130 families in which a case of IGE occurred, a segment on the chromosome 3q26 attracted the researchers' attention. It seemed to contain sequences which predispose IGE. On the corresponding chromosome segment the *CLCN2* gene is also situated, which codes the voltage-dependent chloride ion channel CIC-2. This channel is very widespread in the brain, especially in the neurons, which are inhibited by the neurotransmitter Gamma Amino Butyric Acid (GABA). In the search for relevant mutations, Dr. Heils' team investigated the *CLCN2* gene in 47 families with IGE. The scientists analyzed the 24 exons of the *CLCN2* gene and the neighboring splicing regions. "In three different families we found three different mutations which were inherited together with the epilepsy disease. The mutations either lead to an exchange of amino acids, a too premature stop codon or to atypical splicing," Armin Heils explains. To check whether the mutations in fact impair the function of the chloride channel, expression experiments were performed at the universities of Ulm and Aachen in the research groups of Assistant Professor Holger Lerche and Professor Christoph Fahlke. They discovered that the channel becomes functionless due to two of the mutations. In contrast, the third mutation influences the voltage-dependent opening behavior of the channel. All three mutations probably lead to the fact that GABA can no longer effectively inhibit the affected neurons and/or that the nerve cells can be more easily stimulated. "Only recently, three other research groups found mutations in the GABA receptor in several families with idiopathic epilepsies. The mutations in the *CLCN2*

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gene which we identified are further evidence that the inhibition transmitted by the GABA plays a critical role in epilepsy," Dr. Heils explains.

MOUSE MODEL FOR EPILEPSY

The group of Dr. Dirk Isbrandt at the Hamburg Center for Molecular Neurobiology also belongs to this NeuroNet of the NGFN. Its research interest is a certain group of voltage-dependent potassium channels in the nerve cells of the brain, the M-channels. These channels mediate a potassium current which regulates neuronal stimulation and response patterns. Mutations in the genes coding for subunits of this potassium channel, *KCNQ2* and *KCNQ3*, are the cause of benign familial neonatal convulsions in humans (BFNC). This complicated term denotes a very rare form of epilepsy, which appears shortly after birth. Usually the seizures stop on their own after weeks or months. The Hamburg scientists wanted to investigate in more detail the function of the M-channel in the brain and its role in the development of epilepsy. Christian Peters, a doctoral student in Isbrandt's laboratory, therefore produced mouse mutants which carry a dominant negative mutation in the *KCNQ2* gene. The mutation changes the pore of the potassium channel. "Since a knockout in the KCNQ2 subunit is lethal, we have bred mice that in addition to the wild-type genes also carry the mutated gene," Christian Peters explains. "We wanted to impair the function of the M-channel by assembling the mutated subunits together with the wild-type subunits." Measurements of the M-current showed that this strategy in fact functioned: The amplitude of the M-current was significantly lower in mice with the mutated potassium channel subunit.

Birgit Rau (Heils group)

HYPERACTIVE AND FORGETFUL

The scientists used an inducible system to be able to control the timing of the expression of the mutated gene. "We used the Tet-Off system. As long as the animals received doxycycline, a derivative of tetracycline, in their drinking water, the mutated gene was not expressed." Mice that expressed the KCNQ2 mutant from birth on suffered as adults under frequent epileptic seizures. Moreover, they were extremely hyperactive. In brain slices of these mutants the Hamburg scientists could detect pathological changes in the hippocampus. As comparison, Christian Peters bred mice that also carried the mutated transgene, but in these mice the expression was suppressed during the first weeks. These animals clearly exhibited fewer and less serious seizures, had morphologically normal brains and were not unduly active. "M-currents seem to play an important role in brain development in the first few weeks after birth," the scientist concludes. But M-currents are also important at an adult age: "When we induced the expression of the mutated KCNQ2 subunits first in adult mice, so that their brain was able to develop normally, we detected an overexcitability of the hippocampal neurons in these animals, too, along with reduced memory performance." With their work the Hamburg team proved directly for the first time that M-currents play a crucial role in the stimulation of cellular and neuronal networks, the development of the brain and in cognitive processes. Alongside basic insights, they expect from the mouse model that it will also facilitate the search for new therapies for epilepsy. Dirk Isbrandt says in summary: "Until now there have only been a few animal models suitable for researching the molecular causes of epilepsy. We hope that our mouse model will be a useful tool in the development of novel antiepileptic compounds and therapies."

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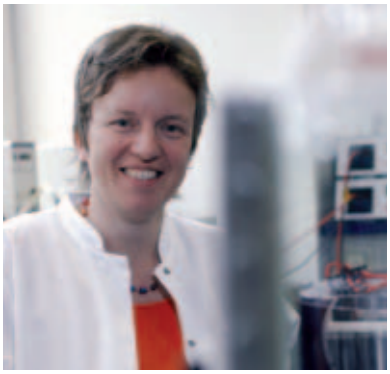
OBESITY – ARE GENES TO BLAME?

Slender, physically fit people smile from the billboards; the magazines advocate diets, healthy nutrition and more exercise. Who doesn't obsess about a few unwanted pounds? But these "problem pounds" seem ridiculously lightweight when compared to people who are obese. Obesity is defined as having a body mass index (BMI) of 30 kg/m^2 or higher. Individuals normally become obese if they have a specific genetic makeup that predisposes them for this condition. In the second funding phase of the NGFN, the network "Obesity and Related Disorders" has received a grant of more than four million euros. Within this network, the research group of Professor Johannes Hebebrand and Dr. Anke Hinney and 17 other groups throughout Germany are studying the genetic causes of obesity. Their aim is to identify genes and gene variants relevant to the disorder to enable subsequent clinical, epidemiological and functional studies. Furthermore, the scientists want to find out if genes involved in obesity are also implicated in comorbidities such as type 2 diabetes, stroke or hypertension.

MUTATION WITH "WEIGHTY" CONSEQUENCES

Mutations in the gene encoding the melanocortin-4 receptor (MC4R) have been shown to contribute to obesity. The MC4R is expressed in the hypothalamus and regulates energy homeostasis and body weight. Both appetite-stimulating (orexigenic) and hunger-suppressing (anorexigenic) factors bind to the MC4R. "Normally, appetite-suppressing factors are likely to predominate at the MC4 receptor," says Johannes Hebebrand, coordinator of the NGFN network. "Knock-out mice lacking this receptor eat more and exercise less than their wild-type counterparts." His own research is currently focused on the *MC4R* gene. At present more than 70 gene variants of the receptor are known. Most of these impair receptor function or even cause a total loss of function. Johannes Hebebrand and colleagues analyzed the actual quantitative effect of these MC4R mutations on BMI. "This impact can easily be overestimated, since in many studies only extremely overweight persons were analyzed to find out whether they carry an *MC4R* mutation or not," he explained. Finally, it is also known that an individual's body weight is influenced by several factors, both genetic and environmental. To study the relevance of *MC4R* mutations for body weight, Johannes Hebebrand and his colleagues analyzed extended family trees (pedigrees) of 22 individuals who harbor functionally relevant mutations. The families were ascertained out of a larger study group of 808 extremely obese children and adolescents. Within the 22 families the researchers determined the genotype and phenotype of 207 persons including 26 obese patients, 43 parents, 22 siblings and 116 more distant relatives. "Our results confirm that mutations in *MC4R* pose a high risk for people to become extremely overweight. Individuals who carry functionally relevant mutations had a significantly higher BMI than their relatives who had no mutations," explains the mathematician Astrid Dempfle. Interestingly, this effect was twice as high in female mutation carriers compared to men. This means that a man 180 cm tall with a functionally relevant *MC4R* mutation weighs approximately 13 kg more than a relative of the same sex who does not carry a mutation; a woman 170 cm tall weighs even 27 kg more than a non-carrier female relative. The scientists noted, however, that even in relatives without the mutation the incidence of being overweight was noticeably high – a strong indication that yet other genetic and social factors contribute to the obesity in mutation carriers. "In the upcoming years more gene variants that influence body weight will certainly be discovered," Johannes Hebebrand adds, "including many that may not have such a pronounced effect as seen for mutations in *MC4R* but that occur more frequently in the population."


Anke Hinney


Jitka Andrä (Hinney group)

GENETIC PROTECTION AGAINST OVERWEIGHT AND OBESITY

However, *MC4R* variants do not always make people obese. In a meta-analysis, statistician Frank Geller and his colleagues were able to show that the variant V103I, which had previously not been considered functionally relevant, has a protective, i.e. "weight reducing" effect. V103I is termed a polymorphism because its incidence in the population is

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greater than one percent. The polymorphism affects amino acid position 103 of the receptor, whereby the amino acid valine (V) is replaced with isoleucine (I). In pharmacological studies no difference between the function of the I103 variant and the V103 wild-type receptor could be detected. Hebebrand's group only noticed the effect of the I103 variant because in a study involving 1,040 parents and their obese children, of 35 parents who were heterozygous for the polymorphism only 10 passed on the I103 variant to their obese children. Based on this result, Frank Geller calculated that the I103 allele is negatively associated with obesity. Subsequently, the V103I polymorphism was analyzed in two large research groups in Augsburg and Essen. Frank Geller merged these data with all published data on this V103I polymorphism in obese and lean individuals. Altogether, more than 7,500 individuals were analyzed. Only due to these large numbers could it be shown that carriers of the I103 variant have a reduced risk to become obese. Frank Geller calculated that an adult man with the I103 variant weighs approximately 1.5 kilograms less than someone who does not harbor this variant. Dr. Iris Maria Heid and her colleagues confirmed this finding in a cross-sectional study of almost 8,000 individuals from the Augsburg region. They likewise showed that the rare I103 allele is negatively associated with overweight and obesity.

CATASTROPHIC ENVIRONMENTAL CONDITIONS

In 1997 the World Health Organization declared obesity an epidemic. More than 50 percent of the German population is considered to be overweight (BMI ≥ 25 kg/m^2). Especially alarming is the increasing number of obese children and adolescents. Environmental factors such as changes in lifestyle are responsible for this increase. Most humans have gene variants which predispose them to develop obesity. During evolution these variants were beneficial. They first became a problem in modern industrialized countries with their never ending supply of easily available, highly palatable and high-caloric food. Also, lack of exercise contributes to this development: cars, elevators and escalators are convenient, and children spend their time in front of the TV or at the computer instead of outside. Even in our working environment sedentary activity predominates. "For people with a genetic predisposition for obesity these environmental conditions are disastrous," Johannes Hebebrand says. Researchers like him have for a long time recognized that the frequent failures in losing weight cannot be traced back solely to a lack of willpower or motivation. But this is highly controversial in light of the general perception that fat people could become thinner if they would only eat less. Obese people who don't succeed in maintaining the weight that they have lost with a huge effort often feel guilty and withdraw socially. "Obesity must be recognized as a disease and destigmatized," he demands. "In this context, the insight into obesity we have gained at the molecular level will make a significant contribution."

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Christel Bonnas (Priller group)

STROKE – HELPING THE BRAIN TO REPAIR ITSELF

A stroke comes like a bolt from the blue. Suddenly one half of your body is paralyzed, you can no longer speak or only see half of what you would normally see. What causes this is a circulatory disturbance in the brain, either because an artery is stopped up or because there is bleeding in the brain tissue. The consequence is impaired circulation: Nerve cells die and can no longer fulfill their function. Cell death takes only a few minutes. "However, the brain is also able to regenerate itself," says Professor Peter H. Seeburg of the Max Planck Institute for Medical Research (MPIMF) in Heidelberg. "After a while patients can partially recover their lost capabilities. Due to the rewiring of the neuronal connections, the surviving nerve cells can partially take over the function of the dead brain tissue. Professor Seeburg and his colleagues in the NeuroNet "Genomic Mechanisms of Functional Recovery from Stroke" are fascinated by these compensation mechanisms. The NGFN researchers want to support the rewiring of the nerve cells and thus keep functional deficits following a stroke to a minimum. But to accomplish this they first have to understand the network. How does our brain go about repairing itself? Which genes are activated while doing this? Which proteins are produced? Which cells step into the breach for their dead neighbors? In the NeuroNet scientists are trying to find out the answers to these questions.

TISSUE DAMAGE REDUCED BY 40 PERCENT

A team led by Dr. Armin Schneider of Axaron Bioscience AG in Heidelberg is studying the granulocyte-colony stimulating factor (G-CSF). G-CSF plays an important role in the hematopoiesis of the myeloid cell line – but apparently not only there. As the researchers proved, nerve cells in the brain express both the G-CSF receptor and also the factor itself. A whole series of indications point to the fact that G-CSF significantly contributes to the elimination of the consequences of stroke. Armin Schneider and his team triggered an artificial stroke in mice by interrupting the blood flow in an important brain artery. Just two hours later the concentration of G-CSF in the brain tissue had risen by more than 100 times, and the number of receptors had also increased. Most apparent were these changes in the brain regions which were adjacent to the area affected by the stroke. Further experiments showed that G-CSF passes the intact blood-brain barrier. This fact gave the researchers the idea to use this factor therapeutically. They injected G-CSF into the mice intravenously following an induced cerebral ischemia. "The success was impressive," says Armin Schneider, who heads the project. "In comparison to untreated mice the size of the infarction area was reduced by more than 40 percent. Moreover, mice who were administered G-CSF developed fewer grave neurological deficits than the other experimental animals." Since this discovery the scientists have also figured out how G-CSF protects brain tissue. On the one hand, the substance prevents the apoptosis of cells in the infarct area. On the other hand, it influences the neuronal stem cells of the brain. These also carry the G-CSF receptor on their surface. Under the influence of G-CSF the stem cells develop further into nerve cells. These can then repair the damage that the stroke has incurred. Armin Schneider is therefore optimistic about being able to help afflicted patients by means of G-CSF. "It could turn out to be the ideal substance to treat strokes and neurodegenerative diseases."

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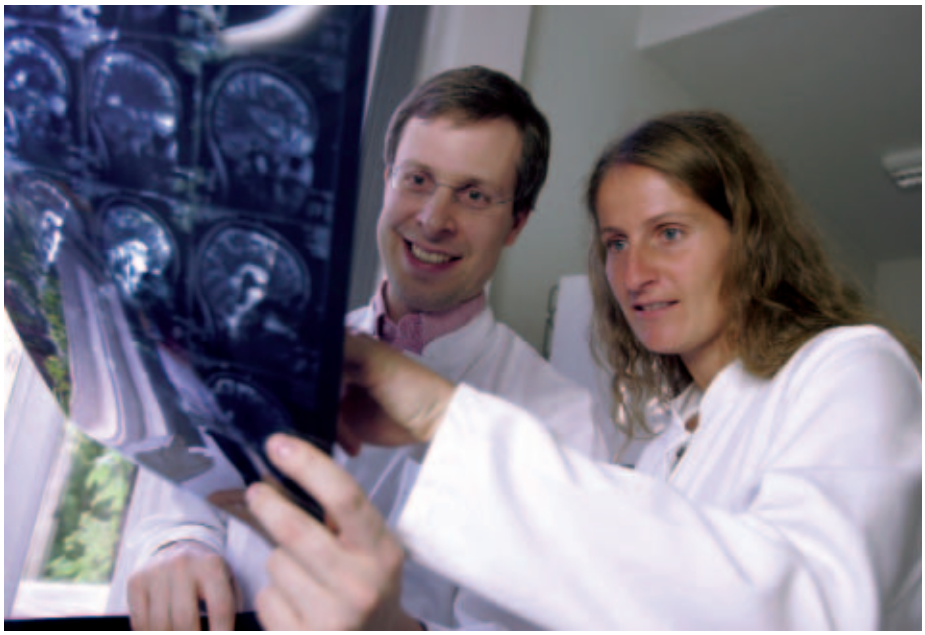
SUCCESS VIA GENE EXPRESSION ANALYSIS

Researchers at Berlin's Charité University Medical Center are also searching for proteins that are involved in the formation of new circuits in the brain. To achieve this, the research team of Professor Ulrich Dirnagl and Professor Josef Priller is analyzing which genes are more strongly expressed in brain tissue after a stroke. They interrupted the blood flow of a rather large area in the brain tissue of mice for two hours.



Josef Priller and Daniela Bermpohl

After reperfusion they performed an analysis of gene expression in the brain tissue. In total, they found 83 transcripts which were strongly expressed during the ischemia phase and 94 transcripts which were less strongly expressed than in the healthy brain tissue. Most conspicuous was an increased concentration of the proteins metallothionein II (MT-II) and – less pronounced – its "sibling" MT-I. The rise of MT-I and -II mRNA expression was relatively rapid and could be detected only two hours after the ischemia phase. In further experiments it became clear that mice in which the genes for MT-I and MT-II had been silenced showed far greater damage to the brain after a stroke than "normal" mice. The animals also suffered from more severe neurological deficits. "Metallothioneins seem to have a protective effect in the case of ischemic brain damage," Ulrich Dirnagl says. By means of serial gene expression analysis he hopes to soon be able to identify additional factors which could be possible target structures in stroke therapy.

BONE MARROW STEM CELLS BECOME NERVE CELLS

Scientists in the Dirnagl-Priller working group, however, are pursuing yet another approach. They are investigating whether bone marrow stem cells can develop into nerve cells in the brain. If this should be the case, by means of a transplantation of these cells the damage to brain tissue following a stroke could potentially be limited. The research team's findings so far appear to challenge current hypotheses about the predetermination of the various stem cell lines. For their experiments the scientists marked the hematopoietic stem cells from the bone marrow with green fluorescent protein and administered the cells to experimental animals intravenously. Ten to fifteen months later two kinds of nerve cells marked with the protein could be detected in the brain of the animals. "Despite the blood-brain barrier, stem cells find their way from the bone marrow into the brain," Josef Priller concludes. "There they apparently can forget their actual hematopoietic determination and transform themselves into nerve cells. It seems that the biological barriers are less impervious than assumed." For stroke patients these permeable barriers mean new hope.

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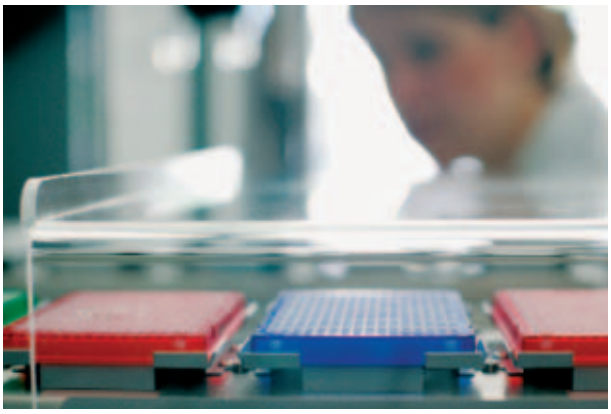
INFECTION AND INFLAMMATION – WHEN THE BODY CATCHES FIRE

The annual wave of flu, SARS, increasing resistance against bacteria – despite immense medical progress, infectious diseases are not yet under control. Worldwide more than 15 million people die of infection every year. Usually the body responds to disease-causing viruses and bacteria with an inflammation, a defense reaction to protect the organism. Inflammation, however, can also occur independently of an infection, as for instance in rheumatoid arthritis. Both aspects of inflammation – acute and chronic – are the subject of investigation by scientists of the Disease-oriented Genome Network Infection and Inflammation. Thus patient-oriented research is being carried out in the areas of sepsis and chronic-inflammatory rheumatic diseases, tuberculosis and parasite infections such as malaria as well as viral infections leading to hepatitis and immunodeficiency (AIDS). The objective is to catalog and categorize disease-specific gene expression profiles and to identify susceptibility genes in order to facilitate the earliest diagnosis possible, to predict the course of the disease and to introduce an effective therapy as rapidly as this can be achieved. In addition, the network supports investigations into the underlying mechanisms of disease using cell- and tissue-cultures, *ex vivo* organ- and animal models, focusing mainly on the innate immune system and in particular the family of Toll-like receptors (TLRs). Via these receptors structural features of the pathogens are recognized and defense reactions are triggered. In order to cope with the plethora of clinical and experimental data deriving from these studies, the areas of data management and bioinformatics, microarray analysis and the bioinformatics core unit as well as a cell-typing and sorting unit are affiliated with the network as so-called "bridging projects" and play a central role. The main objectives within the framework of data management are the linking of clinical findings with data coming from functional genome analysis. An important focus of research is the development of a central warehouse database which merges and interprets all of the data collected in the network.



High-throughput DNA analysis

"Our network combines clinical research and basic research involving the most common infectious diseases and chronic inflammatory rheumatic diseases of society. It provides us with novel insights about the underlying disease mechanisms and helps us to transform these into new diagnosis and therapy approaches. An essential focus of our research is thus investigating natural resistance against infectious diseases. This should give us information as to why not every person gets an equally severe case of the disease and why not every person who has contact with a pathogen gets sick," explains Professor Trinad Chakraborty, coordinator for the network Infection and Inflammation.

SEPSIS DIAGNOSIS AS EARLY AS POSSIBLE

"Severe sepsis and septic shock are the main causes of death in non-cardiological intensive care units the world over. Despite the best possible intensive therapy, the mortality rate of severe sepsis has remained unacceptably high, at about 40 percent, for decades," says Dr. Thilo Menges of the University of Giessen, who is head of the subproject "Clinical Sepsis Phenotypes". His team is analyzing transcription patterns that are associated with the degree of severity of the disease. To accomplish this, the scientists take blood samples of the patients during their stay in the hospital to perform microarray analyses. In their research the scientists focus on three large patient groups: polytraumatized patients, patients with severe pneumonia of the lung and premature babies who were born before the 32nd week of pregnancy. All clinical data, findings and lab parameters of these patients are stored online and can be correlated with the expression data from the microarray analyses. "In the polytraumatized patients the gene expression analyses show clear differences between septic and non-septic patients already at the time of admittance into the intensive care unit," says Thilo Menges. "The gene expression profiles determined in the premature babies show clear differences between artificially respirated infants with congenital sepsis and infants without an infection. Our data from both of these studies suggest predictive diagnosis for sepsis soon after injury or insult. Perhaps they could enable diagnosis of a fetal inflammatory response (FIRS) right at the time of birth."

JOINTS UNDER ATTACK

A further focus of the genome network Infection and Inflammation is on chronic inflammatory rheumatic diseases.

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Thomas Häupl

These include rheumatoid arthritis and spondyloarthropathies such as ankylosing spondylitis (AS). In these diseases the immune system attacks the body's own tissue and destroys it, seemingly without cause. The working group of Dr. Thomas Häupl of the Charité in Berlin is investigating gene expression patterns in blood cells, joint liquid and synovial tissue of patients with rheumatoid arthritis and AS. In cooperation with clinics, the scientists built up a database for this containing the blood, tissue and DNA samples of 500 patients already in the first NGFN funding period. "Over the long term we want to describe gene expression patterns by means of which rheumatic diseases can be diagnosed at a very early stage and the further course of the disease can be prognosticated," Thomas Häupl says. "Up to now the diagnosis has often been made very late after irreversible damage has already occurred." He and his colleagues are therefore currently using gene expression patterns to attempt to find the specific factors that play a crucial role in the early stage of the inflammation.

Already in the NGFN's first funding period the Berlin researchers identified molecular patterns and markers in blood monocytes and synovial tissue which enable a diagnostic differentiation between rheumatoid arthritis and arthrosis. In addition, in both diseases they determined clear differences to healthy tissue in the gene expression. Based on these findings, Dr. Bruno Stuhlmüller, who heads a further subproject of the genome network at the Charité, developed a microarray with the cDNA of 311 genes relevant to rheumatoid arthritis. By means of this array the scientists can classify the degree of inflammation in patients with rheumatoid arthritis and predict how well a treatment would work. Bruno Stuhlmüller now wants to increase the number of genes on the chip and thus further improve the prognostic and prediction possibilities prior to anti-TNF (tumor necrosis factor) therapy. To identify new candidate genes, he is performing preliminary experiments in monocytes, tissue macrophages and T cells. The potentially significant genes detected in the cell cultures are then checked once again by comparing them with gene expression patterns identified by the Häupl team in cells of healthy blood donors and rheumatoid arthritis patients.

PREDICTING TREATMENT SUCCESS

In patients with rheumatoid arthritis, treatment with a monoclonal antibody against the mediator TNF alpha has proved to be very successful. TNF alpha has a central position in pathogenesis because it activates a whole cascade of additional inflammatory cytokines. The therapeutic antibody neutralizes TNF alpha, whereby fewer inflammatory cells penetrate into the joints. However, 20 to 40 percent of the patients do not respond positively to therapy with TNF alpha blockers. "We do not know why this is so. Particularly on the molecular level we have no clear idea of what is actually going on here," says Dr. Andreas Grützkau of the German Rheuma Research Center in Berlin. To elucidate the molecular mechanisms of therapy with TNF alpha blockers, he and Bruno Stuhlmüller are studying the gene expression patterns of patients with chronic inflammatory rheumatic diseases before and after the beginning of treatment. For their expression analyses the scientists utilize highly purified cell populations (monocytes and CD4 positive lymphocytes), which they extract from the blood of patients. "From our earlier studies we know that homogeneous, clearly defined cell populations are considerably better suited for data analysis than heterogeneous populations with an unknown composition," Andreas Grützkau explains. The methods utilized here for cell sorting were already optimized. Soon it will be known why TNF alpha blockers do not work or do not work well for some patients. "Perhaps – even before starting a treatment – we will soon be able to predict the optimal therapy for a patient, based on the activity of his or her genes," the scientists conclude.

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DANGEROUS INTERACTION OF GENES AND LIFESTYLE

When hearing the term "body surface", most people think of the skin. However, the mucous membranes of the intestine, the respiratory passages and the lungs are also barrier surfaces to the outer world and with 300 m^2 are considerably larger than that of the skin (1.4 m^2). These are the barrier surfaces where the body has to deal with many hostile environmental factors. In particular, the mucous membranes of the intestine and the respiratory passages provide an entry portal for pathogens. That is why these tissues have a number of defense mechanisms for building up a barrier against the outside world. These include specialized proteins which fend off invading bacteria, viruses or fungi and also "guards", like the intestinal flora. Our intestine's dense population of bacteria prevents pathogens from being able to settle there. The mucous membranes also have a highly complex and differentiated immune system, the so-called mucous associated defense system.

Lena Bossen (Schreiber group)

DRAGNET SEARCH FOR THE RESPONSIBLE GENES

In the genome network Diseases due to Environmental Factors all research focuses on chronic inflammatory diseases in organs which exercise a barrier function against our environment. Among these are diseases such as allergies, asthma, chronic inflammatory intestinal diseases (Crohn's disease, ulcerative colitis), atopic dermatitis, the lung disease sarcoidosis, psoriasis and chronic obstructive respiratory diseases. To be sure, they affect different organs, but they all have a hereditary aspect to them. Moreover, they all have in common that they can be triggered by lifestyle factors in industrialized societies. In recent decades there has been a great increase in the incidence of asthma and atopic dermatitis in children as well as of chronic inflammatory intestinal diseases – a clear indication that alongside disease genes, environmental factors play a significant role as well. "In our network we are systematically searching for disease genes that together with external influences can trigger disease. We even believe that the seemingly so different environmental diseases are based in part on the same genetic factors," says Professor Stefan Schreiber, coordinator of the genome network. Scientists at the three locations of the network – Kiel, Berlin and Munich – are collaborating closely with the Systematic-Methodological Platforms DNA and Genetic Epidemiological Methods (GEMs). "Alone in the first NGFN funding period of our network over four million single nucleotide polymorphisms (SNPs) were typed," Stefan Schreiber says. These include substantial scientific breakthroughs, such as the identification of the first and second disease genes for Crohn's disease or the discovery of the first disease gene for sarcoidosis.

SARCOIDOSIS – OFTEN NOT RECOGNIZED

Sarcoidosis is an inflammatory disease. Due to the formation of inflammatory lesions, so-called granulomas, it destroys the lungs. Immunologically, there is a hyperactivity of macrophages and CD4 T helper cells. Granulomas can occur everywhere in the body and interfere with the function of the respective organs. Often, however, the lungs are affected. Therefore, scientists suspect that substances from the inhaled air activate the immune system of genetically disposed persons. In Germany an estimated 30,000 people suffer from sarcoidosis. "But most likely there is a high number of unreported cases," says Dr. Jochen Hampe of the University of Kiel, who as young scientist together with his colleague Dr. Ruta Valentonyte discovered the first gene for sarcoidosis. "Due

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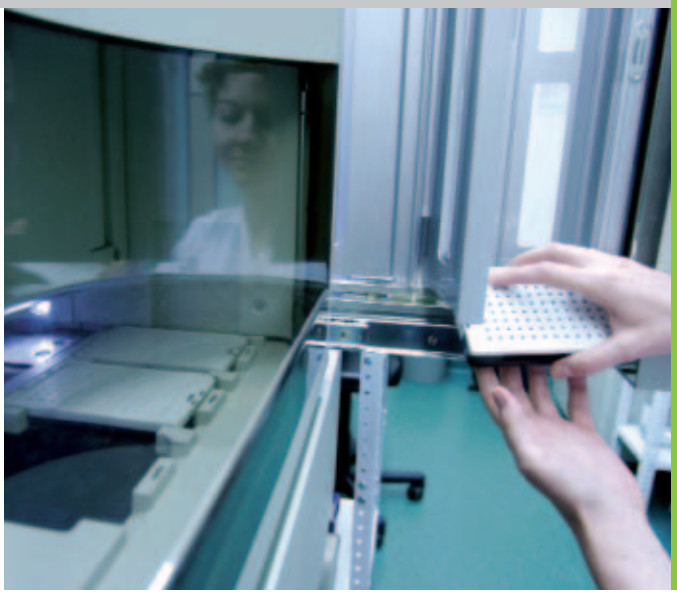


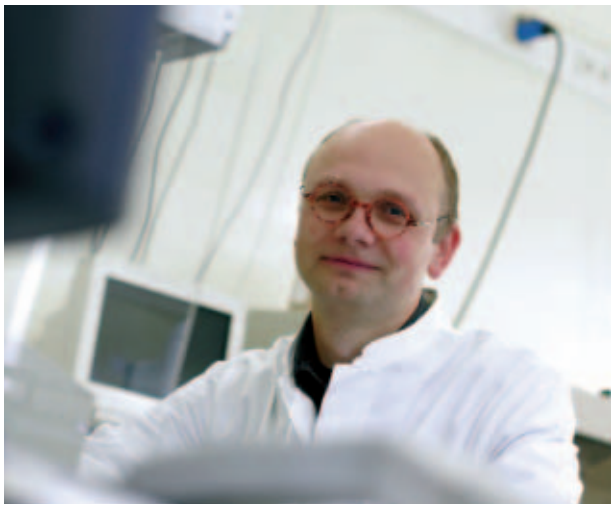
Robotics in genome research

to unspecific symptoms like coughing, joint pain and fever, the disease frequently goes unrecognized."

DISEASE GENE DISCOVERED

Scientists have had indications that sarcoidosis is hereditary for quite some time. There are families in which several family members suffer from the disease, while in other families it almost never occurs. In 2001 Dr. Manfred Schürmann of the University of Lübeck came a step closer to illuminating the genetic causes of the disease when he studied 63 families with known sarcoidosis. He discovered that genetic changes on a segment of chromosome 6 play a role in the genesis of the disease. Together with the Institute for Human Genetics in Lübeck, the Research Center Borstel, the Institute for Molecular Biotechnology in Jena and the Department of Pneumology of the University Hospital Freiburg, the Kiel scientists have now tracked down the culprit: The disease gene's name is butyrophilin-like 2 *(BTNL2)*. Through an exchange of nucleotides a premature stop codon develops in its mRNA. The protein resulting from this lacks important domains for anchoring in the cell membrane. "This loss could lead to the fact that *BTNL2* as co-activator stimulates the T cells too strongly and that thus an autoimmune disease is triggered," Jochen Hampe speculates.

If an allele of the *BTNL2* gene exhibits this nucleotide exchange, the risk of sarcoidosis increases by 60 percent. If both alleles show this variant, the affected persons fall ill with sarcoidosis three times more often than people with "healthy" *BTNL2* variants. As next step the scientists want to elucidate the exact role of *BTNL2*, in order to create a solid basis for new therapy approaches for sarcoidosis. "The discovery of the *BTNL2* gene is a breakthrough for clinical research on sarcoidosis," says Professor Joachim Müller-Quernheim, lung specialist at the University Hospital Freiburg. "Our long-term objective, to predict the course of the disease and the success of therapy, is thus closer to being realized."



Jochen Hampe

NOT EVERY DISEASE HAS ITS OWN GENE

Already during the first experiments to circle in on disease genes for chronic barrier inflammation, the scientists noticed that diseases which attack different organs show considerable overlap. Now the discovery of the disease genes provides certainty: The Munich working group of Dr. Michael Kabesch found out that the gene *CARD15 (NOD2)* and *CARD4 (NOD1)* not only are significant for Crohn's disease, the chronic inflammation of the intestine, but also for asthma, periodontitis and joint inflammation in psoriasis. The Berlin researchers in the working group of Professor Young-Ae Lee report a gene localization for atopic dermatitis which overlaps with a localization for psoriasis. "This will result in completely new possibilities for therapy," Stefan Schreiber says. "The medical implementation of these results means that we must take a holistic view to solve the problem of 'inflammation' across all organ systems."

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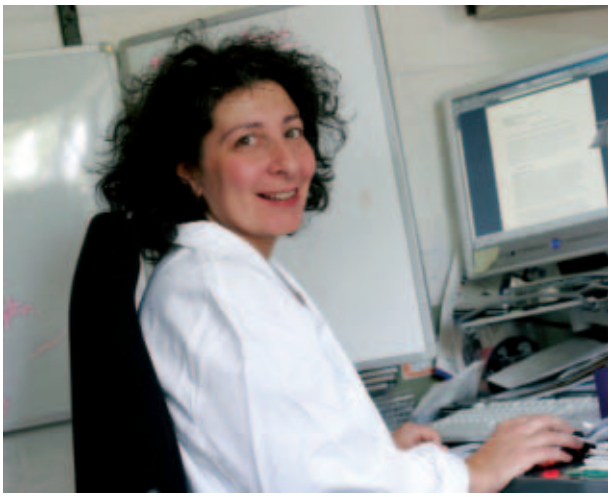
SEQUENCES IN ALL VARIATIONS

Systematic sequencing and comparative sequence analysis – those are two main tasks of the Systematic-Methodological Platform (SMP) DNA. "The sequencing data enable us to analyze phenotypes, genes and promoter elements including their modifications in detail," explains Professor Hans Lehrach, coordinator of the SMP DNA. "Moreover, we can gain insight into evolution by comparing the human genome with other genomes, for instance with the genome of chimpanzees." Besides this, the scientists of the SMP DNA are focusing on understanding the correlations between genotype and phenotype, by means of which they want to identify disease genes and elucidate disease processes.



Marie-Laure Yaspo

THE LITTLE DIFFERENCE

On the DNA sequence level the variation among people is only about 0.2 percent. However, in this seemingly so negligible variability lies the key to understanding complex diseases such as cancer, obesity or hypertension. Scientists worldwide are therefore working on recording the genetic variations within the human genome. Some scientists even describe the search for the variability of the human genome between individuals as the second act of the human genome project. To trace the differences, researchers are pursuing different approaches. In genotyping, for instance, the DNA sequences are systematically scanned for single nucleotide polymorphisms (SNPs). These deviations in single bases are the most common differences occurring in the human genome. Researchers estimate that there are about ten million SNPs in humans. Although many SNPs do not have any effect, some of them increase the risk for getting a specific disease or influence the effectiveness of a drug. Within the framework of the SMP DNA, six genotyping centers have joined together to form the National Genotyping Platform (NGP): the GSF – National Research Center for Health and Environment in Munich under the direction of Professor Thomas Meitinger, the University of Kiel (Professor Stefan Schreiber), the Berlin research groups at the Max Delbrück Center (Professor Norbert Hübner) in Berlin-Buch and at the Max Planck Institute for Molecular Genetics in Berlin (Professor Hans Lehrach, Dr. Sascha Sauer) and the universities of Cologne and Bonn under the coordination of Professor Peter Nürnberg. Together they can provide the NGFN scientists with the entire spectrum of presently known genotyping methods.

TRACKING DOWN DISEASE GENES

"Today, due to modern high-throughput procedures, we can analyze 500,000 SNPs at one time," says Thomas Meitinger of the GSF in Munich. With the aid of genotyping, the scientists in the NGFN have already succeeded in identifying a number of disease genes. For instance, they found sequence variants in the gene for the protein leucine-rich repeat kinase 2 (LRRK2), which are associated with different forms of Parkinson's Disease. Furthermore, they discovered that several SNPs in the coding sequence for the protein FKBP5 are linked with a fast response to antidepressant drugs and a frequent recurrence of depressive episodes. However, genetic variants that predispose for a specific disease are not evenly distributed throughout the world. On the contrary, there are differences evident depending on the population. For instance, a population genetic study in Iceland showed that there are variants both in the gene for the 5-lipoxygenase activating protein (ALOX5AP) and in the gene for phosphodiesterase 4D (PDE4D), which make people with these genetic variants more predisposed to suffer a stroke. A study of the Munich genotyping platform on stroke patients from central Europe showed that in them, too, variants of the gene for ALOX5AP are associated with an increased stroke risk, whereas variants of the gene for PDE4D had no significant influence on stroke risk.

NOTHING LEFT OUT

Dr. Richard Reinhardt of the Max Planck Institute for Molecular Genetics (MPIMG) in Berlin runs the resequencing platform in the NGFN, consisting of the two groups in Berlin and Jena. "In contrast to genotyping we do not focus on individual SNPs when investigating a gene, but sequence the whole gene, or at least all exons," Richard Reinhardt explains. The advantage over all other methods is its unsurpassed accuracy. Resequencing captures all genetic variations – all SNPs but also deletions and insertions. The Berlin research-

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ers already have several achievements to their credit, such as the identification of the molecular causes of kidney diseases and cilia defects that play a role in chronic respiratory diseases. The resequencing platform is continually being expanded and developed to reduce the great outlay of effort and the higher costs involved in comparison to genotyping. "If we develop this here further, one day it will be possible to determine the entire genome of a patient within a short time and with a reasonable amount of effort, so that the costs will also be justifiable," says Richard Reinhardt, describing the future of the resequencing platform.

CHIMP GENOME AS COMPARISON

Dr. Marie-Laure Yaspo is pursuing quite a different approach to decode the functions of the human genome. Within the framework of an international project (the SMP DNA cooperates with the groups from Berlin, Brauschweig and Jena), she is working on the high-resolution sequence of the chimpanzee genome. "Although the chimpanzee is the closest living relative of human beings, there are nevertheless immense differences: for instance with regard to linguistic or intellectual capabilities or the susceptibility for various diseases," the biologist explains, who also conducts research at the MPIMG in Berlin. Within the framework of a German-Asian consortium she was involved in the deciphering of the first chimpanzee chromosome, the chromosome 22. "We assume the comparative analysis of the chimpanzee genome and the human DNA sequence will contribute to discovering disease-relevant genes. But in particular, we will also learn a lot about the evolution of the human genome," she adds. For this the scientists need an unbroken, top-quality sequence of the chimpanzee genome, because it differs from the human genome by only 1.7 percent. Marie-Laure Yaspo is working on the generation of a high-quality sequence of 42 megabases. Most of the segments she has investigated lie on the X chromosome. Numerous hereditary diseases are located on this chromosome, including many forms of mental disability. This is why decoding it is particularly significant. In addition, genomic segments which have been identified as medically interesting by the Disease-oriented Genome Networks in the NGFN are being analyzed in the chimpanzee genome once again by Dr. Yaspo and her team.

INSIGHT INTO THE TRANSCRIPTION MACHINERY

However, within the SMP DNA there are also groups that are not focusing immediately on the whole genome, but rather on its specific segments. For instance, the group of Dr. Michal Janitz at the MPIMG: He and his staff are comparing the activity of promoter sequences in different cell types in order to understand the mechanisms of gene expression and regulation. "We use cell arrays to be able to analyze the promoter regions on a large scale. Our objective is to investigate 3,000 human promoter regions," Michal Janitz explains.
To accomplish this task, plasmids containing a reporter gene driven by a promoter of interest are printed in an array format and then transfected into a number of human cell lines. After the transfection the activities of the promoters can be measured by detecting the reporter gene expression level. Michal Janitz plans to evaluate his data together with the findings from other projects, for example the promoter analysis in mice, in order to trace the global regulation patterns in mammalian cells.

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Cora Mund and Verena Beier

THE INTERPRETATION OF GENETIC INFORMATION

We inherit our genetic make-up from our parents in the form of the DNA sequence. But not all information regarding which genes are to be transcribed and which are not is passed on from one generation to the next. Epigenetics is concerned with such mechanisms "outside" of the DNA sequence. Epigenetic information is acquired in the course of a lifetime. A central epigenetic mechanism is DNA methylation. This process usually occurs on a cytosine (C) which is immediately followed by a nucleotide with the base guanine (G). Although the sequence CG does not occur very often in the human genome, there are certain regions where there is more frequent incidence. They are defined as CpG islands. If such islands are methylated in the promoter region of a gene, it can no longer be transcribed. The CpG islands thus work like a switch. "The DNA methylation pattern represents the epigenetic program of the genome and determines the interpretation of genetic information. Differences in methylation generate differences in the expression pattern," explains Dr. Jörg Hoheisel, coordinator of the Systematic-Methodological Platform (SMP) Epigenetics of the German Cancer Research Center (DKFZ).

DECIPHERING THE METHYLATION CODE

Certain methylation patterns are associated with the appearance of different illnesses. With cancer, for example, a strong methylation of tumor suppressor genes can frequently be observed. "We know that changes in the DNA methylation patterns belong to the earliest and most frequent events in the development of cancer. Nevertheless, still very little is known how these changes arise and exactly what role they play in the degeneration of a cell," says Professor Hermann-Josef Gröne, who oversees the tumor database at the DKFZ in Heidelberg and who also works in the SMP Epigenetics. Furthermore, often there is not any information about how the methylation pattern differs in healthy and diseased tissue. "And this knowledge could be useful for the diagnosis and prognosis of diseases or for the development of new therapies," he says. One reason for the knowledge gap is the lacking methodology: Until now the scientists have not had any tools at their disposal for a genome-wide epigenetic analysis. The researchers at the SMP Epigenetics intend to change this.

MICROARRAYS FOR METHYLATION PATTERNS

Jörg Hoheisel explains the selected approach: "Using a trick, the methylation status of a nucleotide can be represented as single nucleotide polymorphism (SNP). That is why we plan, analogous to the microarrays that search the genome for SNPs, to use microarrays also for a genome-wide and gene-specific analysis of the methylation pattern." The genome researchers then want to correlate the gained information to clinical data and the results of the transcription analyses.

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Using this method, fundamental insights into the role of DNA methylation during tumor development should be possible. In addition, Jörg Hoheisel and his colleagues want to identify methylation patterns that allow diseases to be diagnosed and their prognosis to be assessed. Likewise, the scientists hope that the methylation pattern will also give an indication how effective a medication is.

COMPETENT PARTNERS

The SMP Epigenetics includes both research institutes and private companies. All participants can already look back on several years' experience in the field of epigenetics. One example for the cooperation of research institutions and industry is located at the University of Bonn. Here Dr. Andreas Waha has developed a microarray that contains 7,680 CpG islands of the human genome and which can be used to study epigenetic changes of the genomic DNA. One objective of his project is to extend the microarray so that all CpG islands of the human genome can be analyzed. In particular, he plans to identify all tumor-relevant CpG islands. To accomplish this, he is supported by the Berlin company Epigenomics, which is the leader worldwide in the commercial use of epigenetics. Researchers of the DKFZ, together with the company febit biotech are developing microarrays which are suitable for routine analyses of the methylation pattern. They, too, are focusing on sites that are typical for cancer diseases. DNA methylation analysis has several characteristics which make it an ideal candidate for routine applications. DNA is a relatively stable molecule and thus easier to handle than for example RNA, which is analyzed in transcription studies. Moreover, the regulation mechanisms act much more slowly with reference to the methylation status than regulation mechanisms on the transcription level. The moment the sample is taken is therefore less critical. Another advantage is that the methylation signals "on" or "off" can be digitalized from the very beginning and thus are excellently suited for evaluation at the computer. Within the framework of the SMP, scientists not only undertake a targeted search for significant patterns, but also gather fundamental information about the methylation status of a chromosome, quasi as standard for later studies. This takes place in a coordinated approach between Professor Jörn Walter (University of Saarbrücken), Professor Albert Jeltsch (International University Bremen), Dr. Richard Reinhard (Max Planck Institute for Molecular Genetics, Berlin) and Dr. Matthias Platzer (Leibniz Institute for Age Research – Fritz Lipmann Institute, Jena).

FIRST SUCCESSES

Jörg Hoheisel's team has already taken a first step in establishing genome-wide microarrays in collaboration with the Epigenetics Division of the DKFZ headed by Dr. Frank Lyko. In the scope of a project carried out to prepare their joint work in the Systematic-Methodological Platform, the scientists designed an oligonucleotide microarray with which they can also determine parallel the methylation status of 53 cytosines in the promoter region of a gene. Project team member

Cora Mund

Cora Mund is delighted: "While investigating the methylation status it is possible for the first time to combine a high-throughput with a high resolution." With the previous methods, only the methylation status of merely a few cytosines of a gene could be determined. Next, the Heidelberg scientists want to expand their method: Currently they are working on a chip to ascertain the methylation status of 250 genes which play a role in prostate cancer. "We are convinced that with epigenetic analyses we can provide reliable results that are suitable for routine applications," Jörg Hoheisel explains. "We expect a lot from epigenetics especially for cancer research."

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MOLECULAR SIGNATURES – CANCER LEAVES TRACES IN GENE ACTIVITY

"To combat cancer in a targeted way and with few side effects, we first have to understand the complex dysfunctions in the cells and combinations of cells which underlie this disease," says Professor Annemarie Poustka, coordinator of the Systematic-Methodological Platform (SMP) RNA. In the search for new points of attack for targeted therapies, the analysis of gene expression patterns has proved to be of great value. For this, scientists use microarrays, which enable a fast and exact genome-wide investigation of which genes change their activity in diseased tissue in comparison to healthy tissue. In recent years systematic data on gene expression in tumors and in healthy tissue have been gained this way. Mostly, however, these comparisons have not illuminated the role of these genes in tumor genesis. The subproject "Systematic Analysis of Transcription Networks" of the SMP RNA is therefore aiming to uncover the significance of these genes in the development of tumors. To do this, the scientists are searching for the interaction partners of the genes that are conspicuous in the microarray. Using RNA interference (RNAi), they first suppress the expression of genes of interest in human cells. In the next step they isolate the entire RNA out of these cells and submit in turn this RNA to a microarray analysis. Thus they can recognize which genes modify their activity when the expression of the initial gene is changed through RNAi. "In the ideal case we will identify new connections to already known molecular processes in the cell," hopes Dr. Holger Sültmann, head of the subproject at the German Cancer Research Center (DKFZ) in Heidelberg.

VALUABLE INFORMATION

In the first funding period of the NGFN, Holger Sültmann's working group focused on collecting microarray data on gene expression in tumors of the kidney, mammary glands and the brain. "We worked very closely with clinicians. They contributed tissue samples and their medical expertise to the projects," says Dr. Ruprecht Kuner, post-doctoral fellow in the group. In fact, the scientists were able to assign specific signatures to certain kinds of cancer. How valuable this information can be has been shown with clear-cell renal cell carcinomas: On the basis of the gene expression pattern the researchers can early recognize whether metastases will form in the patient. The physicians thus have the chance to adapt the therapy protocol at an early stage in order to decelerate the formation of metastases as much as possible.

VIRTUAL EXPERIMENTS

In the coming years, 150 of the differentially expressed genes which were identified in the first funding period are to undergo a more detailed analysis, in order to identify further

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tumor-associated genes. Here the focus is on genes whose expression shows differences either between tumor and healthy tissue or changes between various tumor subtypes or stages. SMP RNA scientists are collaborating closely with scientists from the SMP Cell in order to characterize the genes functionally. The work in the laboratory is thus dovetailed with the data analysis of the microarray experiments and the development of new analysis tools. "The computer is the hub of the whole project; here all of the results are integrated so that we can simulate models of the biological networks," says the computational scientist Dr. Achim Tresch. The *in silico* modeling is more than just an attempt to process and manage the flood of data. The computer-calculated models of gene regulation enable the simulation of as many interaction variants as desired. Such experiments, which last only a few seconds virtually, would mean months of work in the laboratory. "The analysis and modeling of the results by computer simulation will enable us to identify networks of gene expression which facilitate the understanding of the pathological mechanisms of human diseases," Holger Sültmann explains.

POSITION DETERMINATION IN THE MOUSE EMBRYO

Professor Bernhard Herrmann of the Max Planck Institute for Molecular Genetics (MPIMG) in Berlin has taken a different approach to finding out how the signal cascades in our body are networked and which dysfunctions cause diseases to develop. "If we want to understand the signal mechanisms that steer the development of organs but that are also involved in the genesis of diseases, we have to identify the genes that control these processes," explains the Berlin scientist. In his subproject he is therefore studying where in the body a gene is active by using whole-mount *in situ* hybridizations (WISH) on mouse embryos. To accomplish this, whole mouse embryos are isolated, processed according to certain techniques and incubated with the cDNA of the candidate gene. "The cDNA only binds in regions where the gene is expressed. Only here does it find the suitable mRNA to which it can bind according to the principle of complementary base pairing," explains Manuela Scholze, who as technical assistant has already performed countless numbers of these *in situ* hybridizations. Professor Herrmann's research group has already investigated over 10,000 cDNAs in the framework of the German Human Genome Project (DHGP). To accomplish this, 9.5-day-old mouse embryos were hybridized. "We have developed WISH into a high-throughput method in order to be able to study gene expression in mouse embryos on a large scale," Bernhard Herrmann says. The Berlin researchers place their data at the disposal of their scientific partners. In this way, they obtain valuable additional information about a disease gene that has just been identified. The gene set which has been analyzed shall now be enhanced. The scientists are concentrating on genes which are involved in signal processes during tissue and organ development and on genes whose expression is disturbed due to human diseases. "9.5- to 11.5-day-old embryos are particularly well suited for these investigations. In this development stage 40 percent of the genes which show a specific expression pattern are involved either in gene regulation or in signal mechanisms," explains Bernhard Herrmann. Moreover, Herrmann's group is focusing on genes that were identified as being of interest in microarrays in the SMP RNA: "We plan to study a total of up to 3,000 genes in 9.5- and 11.5-day-old mouse embryos," Bernhard Herrmann says. They evaluate their data together with the results from the SMP Cell and SMP RNAi and enter them into the international database of the Molecular Anatomy of the Mouse Project (MAMEP). In this way, the Berlin scientist expects to identify additional disease genes, especially for cancer diseases, very soon.

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Ralf Kühn (Wurst group)

OLD MOLECULE BACK IN FASHION – NGFN SCIENTISTS OPTIMIZE RNAi TECHNOLOGY

They had already lost interest in it. Thought they knew everything there was to know about it. But all at once it appeared again on researchers' radar screens: RNA. The reason was the discovery of RNA interference (RNAi). Already back at the beginning of the nineties, scientists noticed that in plants, short RNA molecules could inhibit the translation of genes into proteins. They at first suspected the known antisense phenomenon to account for this "gene silencing". There single-stranded RNA molecules bind to complementary mRNA molecules and thus block transcription. But in experiments with the nematode *Caenorhabditis elegans* the researchers discovered to their surprise that "gene silencing" was considerably intensified when double-stranded RNA molecules were used. This puzzling observation awakened great interest, because the double RNA strands inhibited gene expression very efficiently. Researchers throughout the world worked feverishly trying to understand this so-called RNA interference (RNAi) mechanism. They were successful: Within only a few years scientists were able to explain how this mechanism works (see box). RNAi is a natural process which takes place in many organisms ranging from plants to human beings. Researchers surmise that it is a mechanism to protect the cell against foreign genes, such as parasite or virus genes. The newly discovered and greatly promising RNAi technology will now be further developed by the Systematic-Methodological Platform (SMP) RNAi into a routine method. To accomplish this, the two coordinators Professor Wolfgang Wurst and Professor Tony Hyman have brought together leading German groups in the fields of cell biology, molecular biology, genetics and bioinformatics. "Our goal is to optimize RNAi technology for gene function assays *in vitro* and in the whole organism and to make it available for medical research," Wolfgang Wurst says. The SMP RNAi comprises ten subprojects forming a pipeline for RNAi gene function analysis. This pipeline starts with sophisticated cell culture tests, followed by analysis of mouse embryos and finally culminates in studies of adult knock-down mice. In contrast to the classic knock-out, in which a gene of the genome is made incapable of functioning (null mutation), the knock-down effect achieved with RNAi takes place on the posttranscriptional level and leaves the respective gene undamaged. "RNAi technology is considerably less expensive and time-consuming than the classical knock-out methods. Moreover, with RNAi technology we can even inactivate several genes at the same time," Tony Hyman explains. Furthermore, this technology can be used for gene function analysis in all spe-

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Frozen cell lysates

cies (e.g. rats, productive livestock), for which traditional knock-out strategies are not available due to the lack of embryonic stem cells.

RNAi TECHNOLOGY AS ROUTINE METHOD

In the SMP RNAi three subprojects are optimizing RNAi technology for use in cell culture, whereas two additional subprojects are using RNAi strategies to analyze early stages of mouse embryogenesis. The objective of still other subprojects is to generate adult RNAi knock-down mice as models of human disease. Moreover, the researchers want to produce knock-down mice for candidate genes identified in other NGFN projects. Some of these mice are systematically investigated for relevant signs of disease by the German Mouse Clinic in the SMP Mammalian Models. "We want to develop new standard operating procedures (SOPs) and make them available to all NGFN scientists and the entire scientific community," Wolfgang Wurst explains. In addition, a strong bioinformatics group is included in the SMP RNAi which supports the design of experiments, the evaluation of data and their presentation to the scientific community. Cenix BioScience GmbH, which has many years of expertise in the field of RNAi technology, is also a member of the SMP RNAi: It is active in RNAi technology development and service. In another subproject RiNA GmbH offers workshops and laboratory courses to present the knowledge and insight gained by the SMP RNAi researchers to interested NGFN participants. "The SMP RNAi includes all currently available key technologies for the design and construction of interfering RNAs. Moreover, it will make RNAi technology utilizable for high-throughput screening in human cells and for *in vivo* functional studies in mice," Wolfgang Wurst adds. "We are therefore convinced that we can develop RNAi technology into a routine procedure for *in vitro* and *in vivo* gene function analysis."

RNA INTERFERENCE (RNAi)

RNA interference (RNAi) is triggered by the presence of double-stranded RNA molecules (dsRNA). These are interpreted by the host cell as "foreign" and therefore as "not wanted". First the endonuclease Dicer cleaves these trigger dsRNAs into 21–23 base pair fragments called "short interfering RNAs" (siRNAs). These siRNAs are then integrated into an RNA-Induced Silencing Complex (RISC). The RISC binds to those mRNAs which are complementary to the siRNAs and catalyzes their degradation. If RNAi is used as an experimental tool, the natural RNA interference mechanism can be used to specifically inhibit the translation of desired genes. To accomplish this, the scientists introduce specific trigger RNA into cells or organisms. In invertebrates such as *Caenorhabditis elegans* and *Drosophila melanogaster* this objective is best achieved by using relatively long dsRNA molecules (200–1,500 bp), which contain coding sequences of the target mRNA. However, in mammals the situation is more complicated because here a second mechanism exists which can be influenced by dsRNA: the interferon response. It leads to downregulation of protein synthesis and ends with programmed cell death (apoptosis). The scientists avoid this problem by using siRNA molecules instead. In most cases these evoke strong, reproducible and specific RNAi responses without activating the interferon signal cascade.

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FROM MASS TO CLASS – RESEARCHERS TRACK DOWN DISEASE-RELEVANT PROTEINS

The work of the Systematic-Methodological Platform (SMP) Cell is reminiscent of a filter: At the top the cDNAs go in – at the bottom disease-relevant human genes come out. In between a lot of work takes place, which in the SMP Cell is divided among several groups (www.smp-cell.org). Dr. Stefan Wiemann, at the German Cancer Research Center (DKFZ) and coordinator of the SMP Cell, describes the working groups as modules. Their most important aim is to identify genes and proteins which are involved in the genesis of cancer. "However, our assays also yield results which are relevant for infectious and inflammatory diseases and for the research of neurodegenerative processes." The work of SMP Cell is based on the experience and results of the German cDNA Consortium, the first module of the platform. Since 1996 its researchers have been systematically sequencing and analyzing cDNAs and have identified many previously unknown genes. The cDNA Consortium is therefore significantly involved in an international project to describe all human genes. Furthermore, the scientists of the consortium are building up a cDNA-library. Full-length cDNAs for each gene and each splicing variant from this library are provided to other research projects. "In the meantime the cDNAs which have been sequenced up to now cover 50 megabases of sequence. That is more than the length of the human chromosome 21," Stefan Wiemann explains. The protein-coding regions of cDNAs are cloned in transport vehicles, so-called vectors, out of which they in turn can quickly and conveniently be cloned into other expression vectors. In the meantime about 1,200 cDNAs are available as such "entry clones". Nomen est omen – these clones are the entry point for all further analyses of the SMP Cell.

SNAPSHOTS FROM THE CELL

The module "Protein Localization" already has a long history: "Ever since 1998, in cooperation with the DKFZ, we have been investigating where proteins are localized in the cell," explains Dr. Rainer Pepperkok from the European Molecular Biology Laboratory (EMBL). For this, the scientists fall back on the cDNA clones of the cDNA Consortium. In their investigations they fuse the cDNAs with the green fluorescent protein (GFP) and transfect mammalian cells with it. These then express the fusion proteins generated. "Via fluorescent microscopy we thus obtain snapshots of the protein distribution in a cell," Rainer Pepperkok explains. The scientists investigate each protein in two work steps. In doing so, they tag the GFP onto the C-(3') terminus and also onto the N-(5') terminus. The reason is that at both ends of proteins there are often signal sequences that steer the localization of a particular protein in the cell. The GFP could mask these signals, making the protein lose its orientation in the cell and localize to the "wrong site". Of 567 proteins studied, this was true in 219 proteins. In all cases, the scientists have discovered which of the two ends is responsible for the correct localization by making bioinformatic comparisons with other proteins, among other techniques. With their methods the researchers have localized about 1,000 previously unknown proteins already at the subcellular level. Moreover, Stefan Wiemann and his colleagues observed that protein localization is not a stable factor. It changes, for instance, depending on the cell density. "Cells are dynamic systems," Rainer Pepperkok explains. "To be able to document and analyze these dynamics we will use a new method in the future, 'live cell imaging'." Here a computer-automated camera takes pictures of the cells for several hours, and then from these photos a time-lapse video is produced at the computer visualizing the dynamics of the proteins within the cells.

THE CORE OF SMP CELL

In the next module "Functional Assays", the actual core part of the SMP Cell, high-throughput methods are developed and applied to perform cell-based assays in microtiter plate format. With these the DKFZ researchers investigate e.g. the significance of proteins in cell proliferation, apoptosis or signaling. The results should aid in more exactly analyzing tumorgenesis and inflammation processes. Furthermore, they have established assays with which mitosis, anchorage independent growth and cell invasion can be investigated. Thus, the scientists want to find proteins which play a role in the formation of metastases. "We receive 30 to 3,000 potential disease genes from our partners in the NGFN, especially from the SMP RNA," says Dr. Dorit Arlt, project director in SMP Cell at the DKFZ in Heidelberg. In order to handle the mass of candidate genes that are to be characterized, the scientists first sort the proteins using screens into functional categories. "In doing so, we check whether the promising candidate genes are in fact disease relevant," Dr. Arlt goes on to say. The number of proteins to be more closely investigated then usually shrinks. "But for us there still remains a lot to do," the biologist says, laughing. A whole series of different kinds of assays is needed to find out what role the proteins coded by the cDNAs play in the course of a disease. "Only then will we be able to formulate reliable hypotheses

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regarding function and disease relevance," Dr. Arlt says. "We suspected, for example, that a protein is involved in the signaling pathway of the MAP kinase. The reason is that a small interfering (si) RNA, specific for the gene of this protein, has dramatically reduced the activation of p42/44 MAP kinases." To pursue this hypothesis further, the gene in question was further investigated in DNA replication assays. With success: The protein did in fact stimulate cell division when it was overexpressed. RNA expression profiles ultimately showed that the gene is increasingly expressed in kidney tumors and in metastasizing tumors. It is therefore now a hot candidate for detailed functional and disease-relevant analyses.

BIOINFORMATICS BRINGS ORDER TO THE SYSTEM

A further important module is "Bioinformatics". It manages and interprets the data of the experimental projects. "While developing the high-throughput functional assays, it was clear to us very early on that the data could only be correctly interpreted using statistical methods," says Heiko Rosenfelder, a bioinformatician in the SMP Cell at the DKFZ. "Cells are complex biological systems, and they act and react accordingly. There one can only dream of tidy linear dependencies. Only through the evaluation of high cell numbers does one obtain results with statistic significance." Therefore, after the laboratory work in the SMP Cell, the analysis and management of the experiments take place at the computer. For this, the computer scientists, together with the laboratory researchers, are developing laboratory information management systems (LIMS) that can be quickly and easily adapted to the requirements of the particular experiment. That includes statistical models or meta-analyses for the comparison of multiple experiments. "Due to LIMS our experiments are objective and robust. We try to run as many analyses as we can via the computer to exclude subjective errors as far as possible," Heiko Rosenfelder explains. Moreover, there is an automatic "annotation system" and a half-automatic protein sequence analysis with which protein functions can be predicted. All data of the SMP Cell are stored in a central server, which is accessible to the public. Furthermore, records from other databases are integrated there, in order to directly evaluate the results gained in the SMP Cell, in context with external information.

KEY QUESTIONS

To understand the biological activity of a gene product, certain key questions need to be answered. At what point in time during development and growth is a gene expressed? "This is one of the central questions when one wants to identify disease-related genes," Stefan Wiemann declares. "To cite an example, for this, expression profiles of diseased and healthy tissue can be compared." In which tissues and cell types is a gene expressed and where in the cell is the gene product active? What biological activity does the gene product have and how does the cell react to elevated or lower quantities of this protein? How is the activity of this protein regulated in the cell? In what kind of biological context is the protein active? With which partners does it interact, and what are its substrates? If one answers these questions, one obtains important clues about the biochemical processes in which the protein participates. Taken together, all of these questions and answers are a key to an approach for discovering new drugs.

FURTHER ANALYSIS

The identification of a protein's interaction partners also sheds light on the cellular signal pathways the particular protein is involved in. With the aid of the yeast two-hybrid system (Y2H) and immune precipitation with downstream mass spectroscopy the scientists are therefore investigating protein-protein interactions. In addition, the SMP Cell is collaborating especially closely with the SMP RNA in the NGFN. There scientists are investigating which genes are differentially expressed in normal as compared to diseased tissues. Genes that have become "conspicuous" due to their modified expression in tumors will then be more closely inspected by SMP Cell researchers. "By systematically linking gene identification and high-throughput function analysis we have already traced a number of potential disease-relevant proteins," Stefan Wiemann says in summation. "Now we must analyze in detail what role these proteins play in diseases in order to validate new therapeutic targets."

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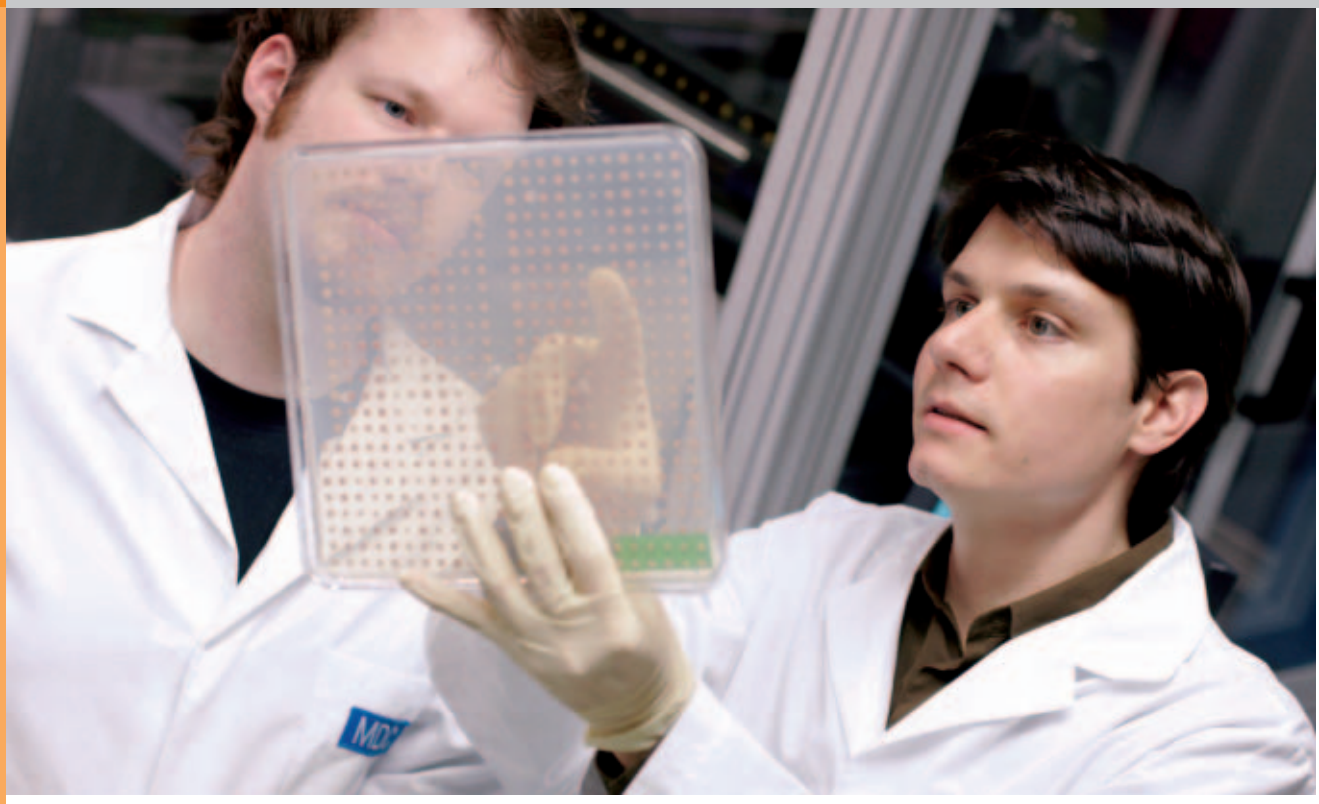

Jan Timm and Ulrich Stelzl

PROTEINS – NETWORKERS OF THE CELL

Inside a large cabinet made of tinted glass, an arm-like robotic device moves up and down, forward and backward. Automatically, it lifts lids off agar plates, dips numerous pointed tips into a microtiter plate and dispenses tiny spots of liquid onto the gelatinous surface of the agar. This is the robotics unit of the Max Delbrück Center for Molecular Medicine (MDC) in Berlin-Buch. "Here, thousands of protein-protein-interactions have been tested in an automated screening approach," Professor Erich Wanker explains. He is coordinator of the Systematic-Methodological Platform (SMP) Protein. "We are employing a yeast two-hybrid system that we have especially adapted to the screening of human proteins. Using it, we can identify protein interactions on a very large scale."

Since the robotics unit was established four years ago, 25 million protein pairs have been tested, and two large protein interaction networks have been generated. One contains 180 interactions that are all connected to the protein that causes Huntington's disease, a severe neurological disorder; the other is a global network of the human proteome consisting of 3,186 interactions between 1,705 proteins. Through these networks, the NGFN scientists hope to gain new insight into the elementary processes in the cell, particularly into their pathological changes. "Knowledge about the interactions helps us find out more about the function of the proteins," says Dr. Ulrich Stelzl, the scientist in Erich Wanker's group responsible for the interaction studies. "By identifying a protein's interactors – its neighbors, as it were – we get clues as to what this protein does. If the interacting proteins are involved in a particular signaling cascade, the odds are high that the protein in question plays a part in that cascade as well."

PROTEIN INTERACTION NETWORKS: A NEW RESOURCE

The ultimate goal of interactomics – the systematic study of all protein-protein interactions – is the generation of a reliable map showing how the whole proteome of a cell connects. This ambitious plan, which can only be realized with the automated procedures established in the SMP Protein, serves several purposes: First, it enables the researchers to discover which cellular proteins interact and share function. Yeast two-hybrid interaction studies try to model what happens between cellular proteins in an experimental set-up and therefore provide many clues about the real situation in the cell. Second, the generated networks are needed and used as resources. By analyzing these networks, it can be found out which proteins form clusters, which proteins act as hubs that interconnect many others and which proteins are fairly isolated. Third, interaction data are collated with data from studies on signaling pathways and disease mechanisms. "If a protein interaction that appeared in a study can be mapped to a pathway that is relevant, for instance, in tumorgenesis,

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Automated two-hybrid screen

we have confirmed the proteins' importance for the disease," Erich Wanker points out. "In this way, interactomics contributes significantly to disease research."

INTEGRATED RESEARCH ACTIVITIES TO FACILITATE THERAPY

The yeast two-hybrid interaction studies, however, are only one part of a larger concerted proteomics strategy intended to yield concrete results to facilitate therapy development for complex human disorders. "The ultimate goal of the SMP Protein is to gain insight about disease-relevant proteins which would enable the development of more specific drugs in a shorter time," Erich Wanker says. To achieve this, research requires clear organization. This is why a unique infrastructure was established that guarantees exchange of data and know-how among groups within the SMP Protein, groups from other SMP, from the Disease-oriented Genome Networks and Explorative Projects. A central office headed by Dr. Patrick Umbach at the MDC makes sure that this exchange functions smoothly. It serves both as an internal control unit for project progress and as a link between the individual laboratories. Regular meetings within the platform ensure close contact among the scientists involved.

ANALYZING PROTEINS, COLLECTING DATA, PROVIDING ACCESS

The production line starts with the preparation of the cDNA clones at the RZPD, the German Resource Center for Genome Research (Dr. Uwe Radelof). From these clones, human proteins are expressed and purified at the Max Planck Institute for Molecular Genetics in Berlin-Dahlem (MPIMG, Dr. Konrad Büssow). At the Protein Structure Factory (PSF) proteins are crystallized and undergo X-ray structure analysis (Dr. Yvette Roske). Next, various approaches are employed to analyze the manifold relationships proteins can form. Protein-protein interactions are studied at the MDC (Dr. Ulrich Stelzl); protein-DNA interactions are analyzed at the MPIMG (Dr. Claus Hultschig). At the GBF, the German Research Center for Biotechnology in Braunschweig, protein-drug interactions are detected with the immediate aim of identifying lead structures (Dr. Ronald Frank). Protein complexes are isolated at the MPIMG from cell extracts and characterized by mass spectrometry (Dr. Bodo Lange and Dr. Johan Gobom). In this way, different methods and techniques permit looking at protein function from a multitude of angles.

Ulrich Stelzl

While these studies are being carried out in the eight experimental labs of the SMP Protein, two further project units are busily gathering and processing the generated data. Professor Olaf Wolkenhauer and his group at the University of Rostock specialize in computer modeling of signaling pathways, while Dr. Ralf Herwig from the MPIMG is responsible for setting up the databases needed for the integration of the results. On his computer screen, Dr. Stelzl zooms into a detail of the interaction map that consists of many hundreds of differently colored dots representing the proteins: "At the end of the funding period, we want all our scientific partners to be able to click on any dot on the map and receive the complete information for that specific protein."

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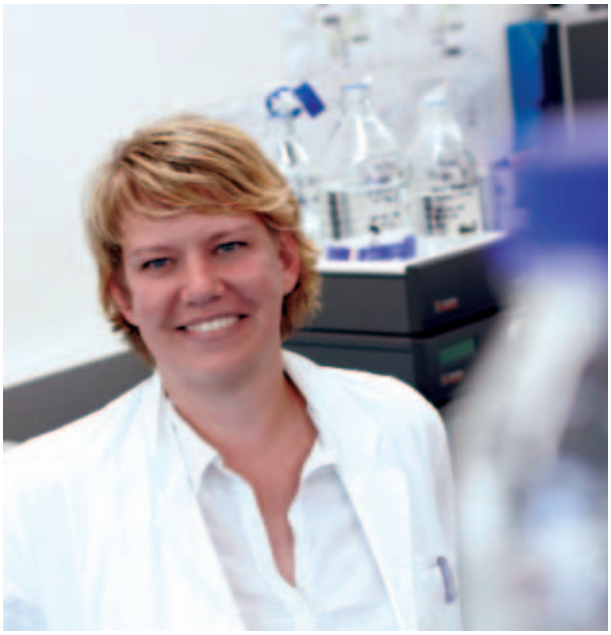
OUR BRAIN – A NETWORK OF PROTEINS

Proteome – a term first coined in 1995 – is the key to understanding complex cellular processes in our body. It can be roughly defined as the protein equivalent of the genome, as the complete set of proteins in a biological system. Protein-protein interaction is involved in all biological processes. The proteome is larger than the genome and more complex: For example, the genomes of the caterpillar and the butterfly are identical – but interaction among proteins is what makes the insect first crawl and then fly.



Katrin Marcus

THE HUMAN BRAIN PROTEOME PROJECT (HBPP)

Scientists of the Systematic-Methodological Platform (SMP) Proteomics in the NGFN are studying the proteome of the brain. In their Human Brain Proteome Project (HBPP) they are systematically analyzing protein expression in the brains of human and mouse. In addition, they are investigating proteins in body fluids such as blood plasma and the cerebrospinal fluid (CSF), which are relevant for the nervous system. The aim of their research is to understand the complex interaction of proteins. Furthermore, the scientists are working on ways to improve already established methods of proteome research. The emphasis of the clinically oriented projects is on neurodegenerative diseases, especially on Alzheimer's and Parkinson's diseases (AD and PD): "We want to contribute to uncovering the mechanisms of neurodegenerative diseases by systematically identifying and characterizing the proteins of the nervous system," explains Professor Helmut E. Meyer, coordinator of the SMP Proteomics at the Ruhr-University in Bochum. "Our work can lead to an enhanced understanding of these diseases. It is even conceivable that on the basis of molecular patterns which characterize the disease, a new classification system may evolve. Besides histological and clinical criteria, this classification would be based on the molecular phenotype, that is, on the mRNA and the protein expression patterns." Ultimately, the new knowledge should improve both the diagnosis and the treatment of neurodegenerative diseases. To accomplish this enormous task, ten academic partners have joined together with the bioinformatics company MicroDiscovery GmbH. In addition, the proteome researchers are collaborating closely with the Disease-oriented Genome Network "Systematic Gene Identification and Functional Analyses in Common CNS Disorders" and the SMP Bioinformatics, RNAi, and Mammalian Models.

CELLULAR INTERDEPENDENCIES – A CELL WIDE WEB

In a subproject of the SMP, Professor Joachim Klose is working to detect the rules of protein networking. "Our working hypothesis is that proteins not only interact within a process or a complex, but that all proteins of a cell have at least indirect contact with each other," he explains. "All of a cell's proteins compete for the same, but limited resources - free space, free water, energy and amino acids." This could function on the basis of a regulatory network encompassing all of the proteins in a cell. Pathological changes in the concentration of a single protein would induce changes in the concentration of the other proteins in a cell, in order to preserve the fine balance between protein quantity and resources. Starting from this hypothesis, the scientists want to show that genetic disturbances and drugs can change the concentration of quite a number of proteins. But not every modified protein may be significant for the disease. Investigations are being carried out on mouse brains with neurodegenerative diseases. "Looking at the proteome of a cell as a whole offers a completely new angle," says Joachim Klose. "With our strategy we hope to learn more about the pathogenesis of neurodegenerative diseases and show starting points for effective treatments." To analyze the functional implications, the scientists are using a high-resolution two-dimensional gel electrophoresis with which they can separate and differentiate 10,000 proteins per gel. "With our experiments we will not only discover disease-specific protein mutations, but also differences which occur in general in several diseases or with aging. Especially proteins which play a central role in the network, e.g. alpha-B-crystalline, which is responsible for the assembly of the proteins, will react to many influences and thus have a nonspecific effect with regard to the disease," Joachim Klose explains. That is why the scientists divide the

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proteins into three groups prior to characterizing them further: specific for a particular neurodegenerative disease, specific for all investigated diseases or nonspecific.

RESEARCH ADVANCES IN PARKINSON'S AND HUNTINGTON'S DISEASES

Results in mice modeled for Parkinson's disease show that Klose's working group is headed in the right direction: The mice lack the protein parkin. Mutations in this protein cause in humans a hereditary and early-onset form of Parkinson. The scientists discovered that the expression of 13 proteins in the brains of diseased mice is significantly reduced. Four of the proteins are directly involved in the oxidative phosphorylation in the mitochondria, whereby this reaction is limited in mice without parkin. As a consequence, the scientists found an increase of harmful reactive oxidative products (ROS) in the brain of the diseased mice. "It is known that ROS can inactivate parkin. It may be that a cycle could develop that leads to the onset of Parkinson," Joachim Klose surmises. "Less parkin reduces the activity of the mitochondria. This generates more ROS which in turn inactivate parkin." His team can also report research advances for Huntington's disease: In the mouse model they found a number of proteins whose quantity diminishes in the course of the disease. Researchers were able to confirm this result by examining post-mortem brain tissue from Huntington patients.

REFINED TECHNIQUES

The research group of Professor Helmut E. Meyer and Junior Professor Katrin Marcus is working on the establishment of an alternative method for 2-D gel electrophoresis. They are developing new multidimensional separation methods for the quantitative and qualitative analysis of complex protein combinations. "At present 2-D gel electrophoresis is the method of choice to carry out comparative proteome studies. But this technology has its limits. It is unable to reliably detect very low quantities of proteins," Katrin Marcus explains. The scientists want to remedy just this disadvantage with a new liquid chromatography based multidimensional separation method. Furthermore, the Bochum team is optimizing techniques to detect posttranslational modifications such as phosphorylation and ubiquitinylation. Such modifications appear to play a significant role in Alzheimer's and Parkinson's disease. "Detecting phosphorylations is a real challenge since the crux of the problem is to detect transient changes that go hand in hand with dephosphorylations," she adds. But already back in the first NGFN funding period the Bochum team, together with the companies Bruker BioSpin GmbH and Protagen AG, succeeded in developing a method to determine the phosphorylation status of proteins. "Now we want to refine and enhance this technology," Helmut E. Meyer says. "Using the technology we have optimized, we hope to identify protein patterns characteristic for Alzheimer and Parkinson in the brain as well as in the blood and the cerebrospinal fluid (CSF)." To achieve this, his team is working with other scientific groups who provide material from mouse brains modeled for Alzheimer's and Parkinson's diseases. Furthermore, they are investigating the CSF and blood plasma of Alzheimer patients and post-mortem brain tissue from Alzheimer's and Parkinson's patients. He stresses, "Since these diseases cannot be traced back to a genetic defect alone, protein expression analysis is essential for understanding the pathomechanisms of Alzheimer's and Parkinson's diseases."

Katrin Marcus, Romano Hebeler and Cornelia Joppich

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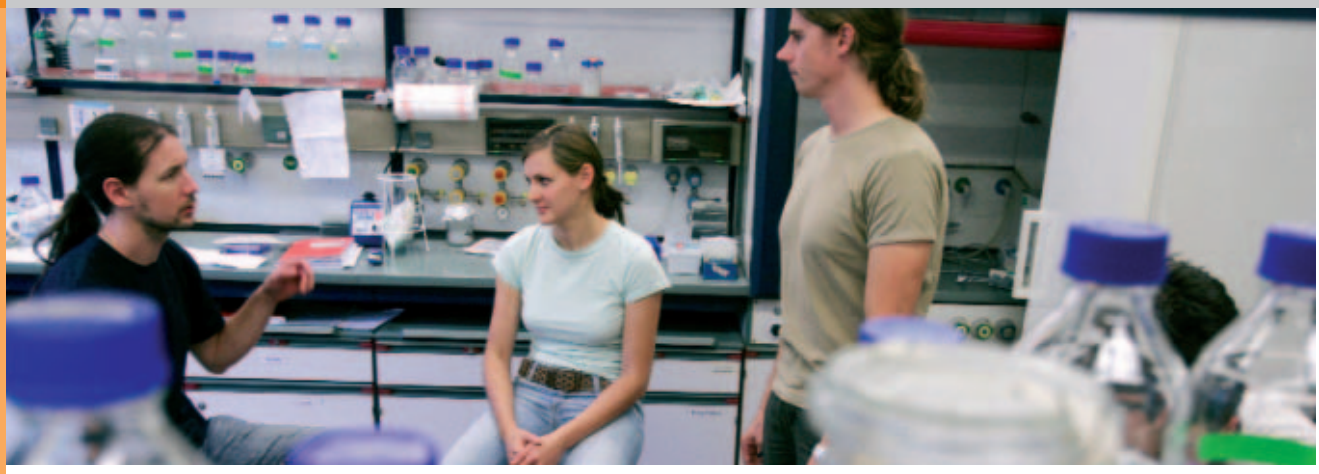

Thomas Schirrmann, Martina Kirsch and Michael Hust

A FACTORY FOR ANTIBODIES

Antibodies – what molecular biologist or biochemist has not yet worked with these precision weapons of the immune system? They are indispensable even for the analysis of genes which have not yet been decoded. The human genome project (HGP) has brought forth a huge number of genes and gene products whose function is still to be determined. Professor Stefan Dübel from the Technical University (TU) Braunschweig estimates that up to 100,000 proteins await their analysis. "Our goal is to establish a pipeline within three years that will produce antibodies for proteomics research in a high-throughput process," Stefan Dübel explains. "This is the only way we can meet the need for antibodies for the functional analysis of genes." The conventional production of antibodies in mice, rats or rabbits often reaches its limits because not every protein evokes an immune response and thus the formation of antibodies. Moreover, this method cannot be completely conducted with high-throughput techniques. Alternative possibilities are provided by *in vitro* selection methods. With their aid the whole process can be automated – from the gene identification number to the finished antibody. Antibodies against non-immunogenic or even toxic proteins can be produced in this way as well. "During the *in vitro* selection process we can control the biochemical conditions," says Stefan Dübel. "Thus, antibodies can also be produced that recognize posttranslational modifications or conformation mutants, e.g. after the binding of a co-factor." The selection method of choice is antibody phage display (see box). Until now, most *in vitro* antibodies have been gained in this way. "Hitherto, the methods and the form of the antibodies have been optimized for the use of human antibodies in therapy," Stefan Dübel explains. "Therefore, antibodies from phage display libraries have not been used for basic research in larger numbers." (see table)

CUSTOM ANTIBODIES

To improve the work with the antibodies, the Institute for Biochemistry and Biotechnology of the TU Braunschweig, the Society for Biotechnological Research, the Max Planck Institute for Molecular Genetics and the German Resource Center for Genome Research in the Systematic-Methodological Platform (SMP) Antibody Factory have pooled their resources and have formed a collaboration. In four closely interlocking subprojects scientists in this antibody factory are working to make *in vitro* selection useful for proteomics research. To accomplish this, they are, for example, concerned with the form of antibodies in their research. The bacterium *Escherichia (E.) coli* used for phage display is not able to produce complete IgG molecules. Small antibody fragments such as single chain variable fragments (scFv fragments) or Fab fragments, on the contrary, can be produced by microorganisms without any problems. Until now, the scientists have generally fallen back on scFv fragments, because the yield of correctly folded and thus functional molecules is greater than with Fab fragments. But the use of scFv fragments can cause problems. On the one hand, they are often not stable enough. On the other hand, they are frequently not compatible with the detection systems of existing assays, which are based on the detection of IgG molecules. In the production of therapeutic antibodies these problems are circumvented by cloning the antigen-binding sequence into other vectors for the production of complete IgG molecules. Proteomics research, however cannot afford to lose time and money with these detours. "That is why we are working on optimizing the use of Fab fragments," explains Dr. Michael Hust, a member on Dübel's research team. "They are more stable and are also usually recognized by secondary antibodies already used in existing assays." He and his colleagues plan to build up different Fab antibody libraries and to compare them with each other. Furthermore, they are testing different methods for panning, i.e. the *in vitro* purification of specific antibodies. To accomplish this, the researchers use ELISA plates, magnetic beads and peptide arrays which are still in development.

SELECTION PROCESS

An additional project is devoted to the preparation of proteins for which the compatible antibody is to be found in the

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phage antibody library. The reason is that researchers often only have the DNA sequence but not the protein. The recombinant expression of cDNAs sometimes delivers the desired protein, but frequently the production in *E. coli* is not easy. That is why scientists are seeking an alternative to proteins to obtain antibodies: synthetic peptides, which in the selection can be used as antigens. "Of course, not all antibodies that are specific to a peptide will recognize the corresponding native protein," says Stefan Dübel. "But the high-throughput method allows parallel screening of several peptides per antigen so that the chances of making a hit are greater. Besides, even an antibody recognizing only denatured antigen is still a useful agent, e.g. for immunoblots or immunohistochemistry." The most recent developments in bioinformatics are contributing to the improvement of epitope prediction. Based on computer prognoses, scientists are trying to produce better targeted peptides for antibody selection. An additional option for protein synthesis is *in vitro* transcription/translation from cDNA libraries. Scientists can use these methods whenever they are not successful in fishing the compatible antibody out of the library using synthetic peptides. "We are trying to integrate these individual components of antibody production into one single pipeline and at the same time to take into account researchers' requirements. In this way the current bottleneck in generating antibodies for proteomics research could soon be cleared, enabling the production of antibodies against problematic antigens," Stefan Dübel emphasizes.

PRINCIPLE OF ANTIBODY PHAGE DISPLAY

Bacteriophages (phages for short) are viruses that adhere to the surfaces of bacteria and transfect their genome into the bacterium. For antibody phage display the genes which code for antibody fragments are fused with a gene for a surface protein of the phages. Subsequently, infected *Escherichia coli* bacteria thus produce viruses which carry antibody fusion proteins on their surface. A mixture of phage clones, all of which carry different antibody genes and therefore express different fusion proteins, is called a phage display library. Using the protein for which one would like to have an antibody, this library is searched. To do this, the protein is coated onto a firm surface (such as magnetic beads or microtiter plates) and incubated with the phage. After several washing steps only phage with the specific antibody fragment remain bound to the immobilized protein. They can be eluted in the next step. This technique of *in vitro* selection via the binding activity is termed "panning". Normally, two to five such panning rounds are performed before individual phage clones can be isolated.

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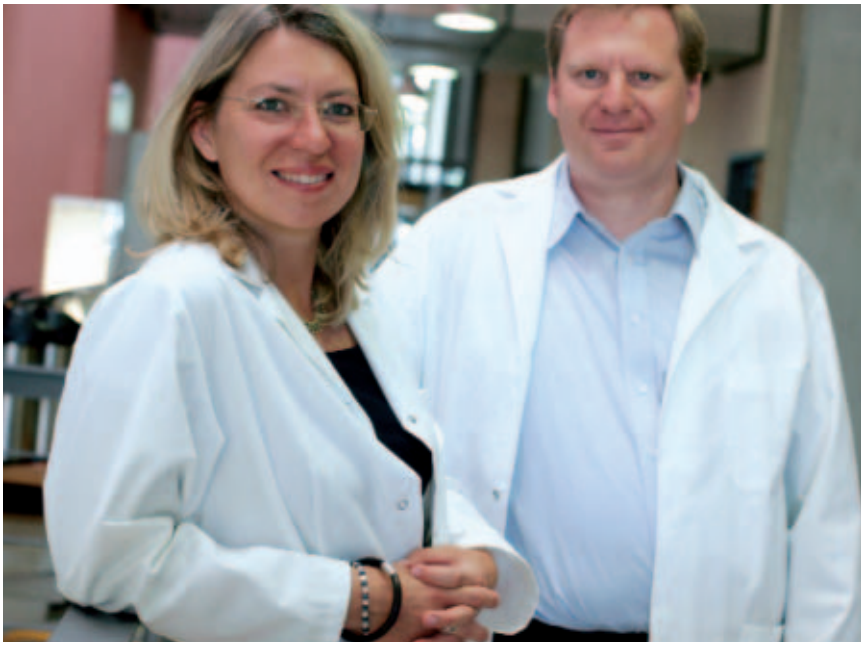
COMPARISON OF THE REQUIREMENTS OF *IN VITRO* SELECTION SYSTEMS FOR ANTIBODIES IN THERAPY OR PROTEOME RESEARCH

APPLICATION	THERAPEUTICS	PROTEOMICS
ANTIGENS	• few • known and available • well characterized • production cost not important	• large number • mostly unknown and not available • unknown characteristics • production costs limiting
SELECTION PROCESS	• arbitrary number of selection rounds • cost of the actual selection relatively unimportant • optimized for maximal success rate • re-engineering of the antibody format essential	• number of selection rounds should be minimal • optimized for low selection costs • maximal success rate not primary goal • avoidance of re-engineering of every individual antibody clone
PRODUCT (ANTIBODIES)	• optimized for minimal immunogenity • optimized for good pharmacokinetics	• optimized to be robust • optimized for maximal compatibility to established assays

A CLINIC JUST FOR MICE

At the age of five weeks the process begins: In the German Mouse Clinic at the GSF - National Research Center for Environment and Health in Neuherberg the mouse mutants are given thorough medical check-ups. First the clinic staff weighs and measures the mice and looks for abnormalities. Then all of the results and the birth date are entered into a database. But that is not all: The mice will progress through 14 stations in the next four months. The German Mouse Clinic (GMC) is a diagnostic clinic in which genetically modified mice are systematically phenotyped. Behavior and lung function are included in the examination modules along with neurology, cardiology and allergies – in the screen no important area is left out. "Hardly any patient is examined as thoroughly as our mice," says Professor Martin Hrabé de Angelis, director of the GMC and coordinator of the Systematic-Methodological Platform (SMP) Mammalian Models, to which the German Mouse Clinic belongs. "Our goal is to find mammalian models for genetically determined human diseases in order to understand them better." For that reason, the SMP Mammalian Models covers the entire spectrum of mouse genetics, ranging from the creation of genetically modified mouse lines, phenotyping, to the archiving of mouse lines. "Creating mouse mutants is one aspect," Martin Hrabé de Angelis explains. "But most important is the subsequent thorough diagnosis. Only when we have understood everything down to the last detail about what happens in the mutants can we elucidate gene functions with medical and biological relevance." The German Mouse Clinic is therefore an essential element of his scientific concept.



Valérie Gailus-Durner and Helmut Fuchs

SCHEDULED DIAGNOSIS

Dr. Valérie Gailus-Durner and Dr. Helmut Fuchs, the coordinators of the GMC, are responsible for ensuring that everything in the mouse clinic runs smoothly. They organize a detailed schedule according to which the individual mouse lines advance through the different investigation modules in a weekly rhythm. "We have taken special care that the tests which are performed on an animal do not interfere with each other. In this way we prevent false results," Valérie Gailus-Durner explains. As a worldwide unique offer, the mouse clinic established this workflow to be able to accept mouse lines of other scientific working groups for investigation. Every two weeks these "foreign" mouse mutants, which are only a few weeks old, are delivered. First of all, the new arrivals have two weeks to get used to their new environment – the food, the smells, the sounds and their keepers – before they are examined. "All mice should have the same situation at the beginning, so that we can compare them with each other," explains Helmut Fuchs.

240 PARAMETERS PER MOUSE

The mouse clinic cooperates with numerous specialists in performing the multifaceted, comprehensive examinations. "Without the close collaboration of clinicians and genome researchers our project would not be conceivable," Valérie Gailus-Durner says. Thus, for instance, neurologists of the Ludwig Maximilian University in Munich test reflexes and muscle tension. At the Technical University Munich blood specimens of mice are screened for infectious and autoimmune diseases. A working group of the NGFN Cardiovascular Diseases Network is presently building up the cardiology department. To make a diagnosis, the scientists use devices like in a normal clinic: electrocardiogram, X-ray and ultrasound devices and blood analysis machines. The only difference is in the size of the machines. For example, a micro-MRI machine was developed especially for mice. After completing the primary screening, the scientists can refer to 240 different parameters. With this data they can judge whether

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Mouse X-ray

the particular mouse line represents a phenotype that is similar to a human disease. Since the GMC was established, the Neuherberg scientists have already examined forty genetically modified mouse lines. "In almost all of them we found numerous deviations in comparison to the genetically unmodified mice," Helmut Fuchs explains. For example, the *Cra1* mouse mutant, a model for hereditary degenerative diseases of the motor neurons, is one of the success stories. The mouse line was discovered with a simple neurological test: When the scientists held up the mouse, the back legs cramped. With increasing age the motor abilities then deteriorated in this mouse line. A single amino acid exchange in the intracellular transport protein dynein causes the motor neurons in the medulla to degenerate. But even mouse lines that have existed for years are put through a thorough medical check-up once again. Only recently this effort was rewarded: In an important cytoskeletal component in a ten-year-old mouse line the scientists detected a pronounced immunological phenotype. Until then, attempts to find out the effects of this mutation had been in vain. Another example is a mouse mutant that represents a model system for Down's syndrome. In these mice, which are difficult to breed, GMC scientists not only detected immunological and hematological effects that are known in humans as well, but also changes in the eye and in behavior.

THE ART OF FREEZING

But how can a scientifically relevant mouse line be preserved? It would involve too much effort to always keep on breeding the lines, and it would also be too risky. Therefore the scientists utilize cryopreservation: Sperm or embryos of mutant mouse lines are frozen in liquid nitrogen. Then, as needed, they can be thawed, revitalized and investigated further. However, not all research institutes master this intricate technique, which requires a high degree of technical know-how. That is why the European Mouse Mutant Archive (EMMA) was founded, which offers all scientists the preservation of their mouse lines free of charge. Hrabé de Angelis is the director of EMMA and coordinates this unique archive, which is comprised of seven institutes in six European countries. All work processes – freezing, medical examinations, handling, and the transport of both the living mice and the frozen material are meticulously regulated in standard operating procedures (SOP). Consequently, the work of EMMA meets the highest quality standards. At the GSF in Neuherberg mainly sperms are frozen; the partner institutes in England, France, Italy, Portugal and Sweden have specialized in embryo freezing.

With its comprehensive concept the GMC has set a new standard in mouse phenotyping. Both in the US and in Asia scientists are already building up mouse clinics according to the same concept. In Neuherberg work is already going on to expand the GMC. Tests are planned with changed environmental conditions: The mice will be exposed e.g. to precisely defined pathogens or allergens for a certain time. Six new examination units and an MRI tomograph for mice are planned for this project.

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MOUSE GENES CAUGHT IN THE TRAP

An important mutagenesis project within the SMP Mammalian Models is the German Gene Trap Consortium (GGTC). The researchers of this project apply different genetic tools to decode the function of genes. Using gene trap technology, they are able to randomly mutagenize genes of the mouse genome very efficiently. The bases of this approach are mouse embryonic stem (ES) cells which are transfected with especially designed gene trap vectors. "Gene trap technology is at present the most efficient validated mutagenesis approach for functional gene analysis", explains Professor Wolfgang Wurst, coordinator of the GGTC. "We perform our functional analyses in mice because they are very similar to humans with respect to embryonic development, physiology and behavior. In addition, 99 percent of all human genes have orthologs in the mouse genome. Moreover, many research findings in mouse disease models are relevant to the elucidation of the molecular cause of human disease conditions."

Mouse under investigation

TRAPPERS AT WORK

In gene trap mutagenesis, a vector integrates into a gene, thereby disrupting its sequence. As a consequence, the protein coded by the respective gene can no longer be formed, and this can have serious effects in cells of the organism. Initially, researchers assumed that gene trap vector integrations occur at random throughout the genome. However, it became apparent that each vector type has its favorite genome integration sites. Thus, to achieve saturation mutagenesis, a whole repertoire of retroviral and plasmid vectors needs to be used. First-generation gene trap vectors have in common that they contain a promoterless reporter/selector gene cassette. As soon as the vector inserts into an active gene, this reporter/selector gene is expressed via the control elements of the trapped endogenous gene, which can be easily visualized in the entire organism. In this way the gene itself announces its disruption and place of activity during embryogenesis and in the adult.

In some cases, however, gene trap integration cannot be detected. For instance, when the gene trap vector integrates into a gene that codes for a secretory protein, the reporter/selector protein will – just like the original protein – be excreted from the cell, thereby preventing its detection by *in vitro* selection. For these classes of genes, the GGTC has developed a special secretory gene trap vector which allows the detection and identification of a single peptide containing genes: U3Ceo. This vector contains a transmembrane peptide which causes the reporter protein to be retained on the inner side of the cell membrane, thus remaining selectable and detectable. With the aid of this secretory gene trap vector, mutations in ligands and transmembrane proteins can be

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detected approximately five times more efficiently than with "classical gene trap vectors". Moreover, the GGTC researchers have constructed new, conditional gene trap vectors which allow the mutation of genes in a time- and space-controlled manner. This conditional mutagenesis approach is very versatile because it can model human disease conditions more precisely – for example an adult-onset form of Parkinson's disease which affects specific neuronal populations in the adult brain.

AN EFFICIENT METHOD

Since its foundation the GGTC has already produced 22,828 mutated mouse embryonic stem cell lines. "For 16,862 of these clones we could determine the gene trap vector integration site in the genome and identified the disrupted gene. This reflects how successful these strategies are," says Thomas Floss, who is a member of the Wurst research group. In this process, typically some genes were hit several times, but nevertheless 3,779 different genes have been mutated,

The German Gene Trap Consortium publishes all sequence data of its gene traps at **www.genetrap.de**, on the websites of the International Gene Trap Consortium (**www.igtc.org.uk/cgi-bin/advanced_search**, select 'GGTC' as cell line source prior to clicking on search) and of the National Center for Biotechnology Information (NCBI; **www.ncbi.nlm.nih.gov**, in 'nucleotides' search for 'gene trap GGTC' to obtain a list of the GGTC genes) as well as on the Ensembl website (**www.ensembl.org/Mus_musculus**, enter gene of interest and then the DAS source: select gene trap). Gene traps of the GGTC and of other origins can be found at **ftp://ftp.ncbi.nih.gov/genomes/M_musculus/GeneTrap**.

among them 279 genes that are associated with human diseases. "Besides, we detected 173 integrations in genes that had not been annotated before. Fifty-two integrations took place in sequence segments that apparently code for regulatory RNAs", he adds. Altogether, the GGTC scientists have already mutated about 15 percent of all mouse genes: "With our project we have created the largest publicly accessible library of mutated mouse embryonic stem cells worldwide. Already several hundred mouse models have been established from our mutant ES cells to investigate gene functions *in vivo*", Wolfgang Wurst says. Among them, many interesting mouse mutants with disease-relevant phenotypes were created: for instance, a mouse line with a mutation in the Nephrin *(Nphs1)* gene. *Nphs1* codes for a structural protein that plays a significant role in urine filtration in the kidney. Nephrin (trap/trap) mice excrete an increased number of proteins with their urine and die soon after birth. This so-called proteinuria is an inherited kidney disease which is frequently found in the Finnish population. Another example are mice that bear a mutation in the gene coding for the latent transforming growth factor beta (TGF-beta) binding protein 4. This protein is a component of connective tissue microfibrils and regulates the deposition and activation of TGF-beta. Mice in which both alleles of this gene are disrupted develop severe heart muscle disease, colorectal tumors and lung emphysema. Furthermore, mutants have been established in *DJ-1* which is a candidate gene for Parkinson's disease. Some of these established mouse mutants are being studied in the German Mouse Clinic, which is also part of the SMP Mammalian Models. "Thus, we are in a position to generate large numbers of mutations and analyze the role of these particular genes in development and adulthood and their potential contribution to disease phenotypes," Wolfgang Wurst explains.

INTERNATIONALLY RECOGNIZED CONCEPT

The GGTC is currently composed of four collaborating research groups: the groups led by Professor Wolfgang Wurst and Dr. Jens Hansen at the GSF - National Research Center for Environment and Health in Neuherberg, the research group of Professor Harald von Melchner at the University of Frankfurt/Main and the team of Professor Patricia Ruiz at the Charité in Berlin. The GGTC was significantly involved in the founding of the International Gene Trap Consortium (IGTC) whose members include BayGenomics, Berkeley, CA, USA; the Sanger Institute, Hinxton, UK; the Fred Hutchinson Cancer Research Center, Seattle, WA, USA; the University of Manitoba, Winnipeg, MB, Canada and the Mount Sinai Hospital, Toronto, ON, Canada. This international consortium is involved in a worldwide effort striving for complete mutagenesis of the mouse genome and endeavoring to provide animal models for every human disease.

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BIOINFORMATICS – WHAT THE DATA REALLY MEAN

Munich, Spring 2005. In seminar room 5 in the Grosshadern Clinic, 26 young scientists are sitting engrossed in their work at computer terminals. In four days the participants are to learn as much as possible about the statistical analysis of DNA microarray data. On this Wednesday morning the topics are molecular diagnosis, classification by nearest shrunken centroids and support vector machines, and model assessment and selection.

Elena Reiffel, Stefanie Scheid, Florian Markowetz and Stefan Bentink

"Just a few years ago everything revolved around questions of basic research," says Dr. Rainer Spang of the Max Planck Institute for Molecular Genetics (MPIMG), in his introduction to the third course day. "Today the main concern is utilizing the molecular data for the diagnosis and therapy of patients." The data analysis often makes decisions necessary that directly affect the patient and must therefore be made very carefully. However, it is still uncommon for a medical doctor or biologist to master algorithms, plot analyses and perform hierarchical clustering. "That is why we decided to offer seminars on selected topics even back in the first NGFN funding phase," says Dr. Florian Markowetz of the MPIMG in Berlin. The courses in microarray data analysis take place four times a year, twice in Munich and twice in Heidelberg. All NGFN members are eligible to register – and if there are any openings left – scientists from other institutions can register as well. "Ideally the participants should be familiar with at least one programming language. However, it is especially important for them to refresh their knowledge of statistics prior to the course, because without statistics you cannot evaluate data," Florian Markowetz recommends. "People interested in participating can find a number of links to reference literature and software to help get acquainted with the topic at **www.compdiag.molgen.mpg.de/ngfn/**.

THREE BIG "I's" – INTEGRATE, INTERPRET AND INFORM

The courses on the analysis of microarray data are part of the module "Service, Training, Consultation and Quality Management" of the Systematic-Methodological Platform (SMP) Bioinformatics. They are only one of many activities which have been initiated to transfer know-how to the partners of the NGFN. The scientists also develop software programs, for example, and provide consulting services for intricate problems. Apart from that, they work closely with numerous projects from the Disease-oriented Genome Networks and help with statistical and mathematical problems. "In the previous NGFN funding phase we were involved in more than 30 projects from all of the clinical areas," says Professor Roland Eils of the German Cancer Research Center (DKFZ) in Heidelberg, who coordinates the SMP Bioinformatics. However, the achievements of the SMP Bioinformatics extend far beyond specific support for individual projects. Besides the module "Service, Training, Consultation and Quality Management" the bioinformaticians are concerned with data management, the standardization of data and data analysis for the entire NGFN. "We speak of the "3-I" approach: integrate, interpret and inform," Professor Eils explains.

iCHIP

"Integrate" is a keyword for data management, an area in which bioinformaticians conceive, develop and install databases and standards for the various NGFN findings. "A complex research network like the NGFN can only work efficiently when all of its members use the same vocabulary and can also access the results of the other groups," Professor Eils explains. An example is the database iCHIP. It was conceived and introduced specifically for clinical research, already back in the last NGFN phase. iCHIP helps scientists manage their findings from investigations with DNA chips, Affymetrix chips and molecular cytogenetic experiments and to link them to clinical data. iCHIP is already firmly established in several Disease-oriented Genome Networks. Next, Roland

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Eils and his colleagues want to extend iCHIP so that findings from cellular assays can also be combined with data from RNAi experiments, proteomics research, matrix-CGH and tissue microarrays (TMA). "Clinical research is changing constantly. New methods are being established and thus new data is generated," Roland Eils explains. "That is why generally valid standards must continually be developed further." To always meet the current quality demands, the SMP Bioinformatics works closely with international committees, for instance with the European Bioinformatics Institute.

INTERPRETING DATA CORRECTLY

Dr. Jörg Rahnenführer of the Max Planck Institute for Informatics in Saarbrücken is active in the field of data analysis. Together with his colleagues, he works out the bases for the statistical analysis of microarray data. In addition, as a mathematician he is involved in clinically oriented projects. Thus, in collaboration with medical doctors from the University of Düsseldorf, the Charité in Berlin and the Saarbrücken University Clinic, Jörg Rahnenführer has developed a prognostic marker with which the survival time of tumor patients and the period until a relapse can be estimated. Such time predictions are very important to classify a tumor and to select the suitable therapy," says Dr. Bernd Wullich of the Clinic for Urology and Children's Urology in Saarbrücken, who is significantly involved in the project. Up to now, physicians have primarily relied on clinical and histological parameters in assessing a tumor. But interest in genetic markers is increasing. With their help, scientists are hoping to predict the course of cancer in a patient much more accurately. "A considerable number of research findings already exist that are based on individual genetic mutations in order to assess a tumor," Jörg Rahnenführer says. "But actually we need more complex analyses that take all genetic changes in the course of a cancer disease into consideration. Moreover, these analyses must be capable of evaluating the probabilities for possible disease courses." Together with clinicians, he developed a method to calculate this, the so-called genetic progression score (GPS). Genetic changes that have already been recorded are also included in the calculation of this score. "GPS allows us to estimate how the tumor, with the greatest probability, will develop in the patient," Bernd Wullich explains. GPS has already proved to be a suitable prognostic marker for both glioblastomas and prostate cancer to estimate the further course of the disease. Since these are two tumor types with quite different genetic backgrounds, the scientists are convinced that their approach can be applied to other kinds of cancer.

"The primary purpose of our SMP is to simplify the utilization of data generated in the NGFN initiative. We want to facilitate the transformation of data into biomedical knowledge. Our platform has the infrastructure and know-how for this 'data to knowledge' transfer."
Professor Roland Eils

Back to Munich. At 5:00 p.m., after seven hours crammed full of exercises, lectures and discussions, the scientists turn off their computers. Many a head is spinning, but most of the participants seem satisfied as they leave the Grosshadern Clinic. Anja Weigmann from Hannover is also happy that she took the time for this course: "For me, today was very rewarding. This afternoon we evaluated and discussed my data, which I brought with me from Hannover because we simply had not got any further. Now, at last, I have reliable results."

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ON THE WAY TOWARDS A GENETIC EPIDEMIOLOGY

The mechanisms of major common diseases remain elusive – even genetic researchers have not yet really been able to find their causes. And no wonder: In complex diseases like cancer, cardiovascular diseases and allergies hundreds of different genes interact with each other. In addition, environment and lifestyle also play a role. To fully understand this interaction it is not enough to study a few affected persons – large sample surveys and the most advanced high-throughput analysis are necessary. Usually, thousands of people must take part in the relevant studies. It has just recently become possible to carry out genetic analyses on this large scale and to correlate the results to environment and lifestyle variables. These recent advances have greatly increased the demand for expertise in the etiology, distribution and control of human diseases in groups of relatives and – with the inherited predisposition to diseases – in populations. In the NGFN this genetic epidemiological expertise is provided by eight research groups located at universities or research institutes from all over Germany (Berlin, Bonn, Göttingen, Heidelberg, Kiel, Lübeck, Marburg and Munich). Coordinated by Professor Max P. Baur (University of Bonn), they form the Systematic-Methodological Platform (SMP) Genetic Epidemiological Methods (GEMs) and closely cooperate with the Disease-oriented Genome Networks and the high-throughput genotyping platform to further promote research on complex diseases. But also new methods are needed. To accomplish this, four specialized research modules within the SMP GEMs work on quality improvement and data standardization (Module A), the development of new statistical methodologies to analyze an ever increasing complexity of genetic data (Module B), appropriate training programs in genetic epidemiology (Module C) – or the two large bio-database projects (Module D).

BIO-DATABASE IN GERMANY'S FAR NORTH

The first project is coordinated by the gastroenterologist Professor Stefan Schreiber and the bioinformatician and statistician Michael Krawczak at the University Clinic Kiel. The name of the research project: PopGen (Population Genetic Recruiting of Patients and Control Groups). The goals of the Kiel researchers are ambitious. Approximately 25,000 patients in the northern part of the state of Schleswig-Holstein who suffer from one of twelve chronic diseases are to be contacted, and data is to be gathered on them. Stefan Schreiber, who is at the same time one of the spokespersons for the NGFN, says: "Individual genes that play a role in these diseases are already known from smaller studies. Within the scope of PopGen and using a larger patient collective, we want to elucidate how great their influence on the course of the disease actually is. Moreover, we hope to gain information about how often these disease-causing gene variants actually occur. This objective can only be achieved through a close cooperation of clinicians and methodological scientists, just like we have in PopGen." To enable a comparison with the "normal population", the data of 25,000 randomly selected healthy inhabitants of Schleswig-Holstein will be collected. A sample of 30 ml of blood will be taken from every participant of PopGen. Diseased patients will also answer questions about their lifestyle and possible risk factors. It is planned to repeatedly question half of them briefly about the course of their disease every six months.

Other project groups in the NGFN can turn to PopGen with special requests. Stefan Schreiber explains, "For instance, when colleagues want to know how many people bear a certain variant of the gene X that plays a role in asthma, we carry out the relevant analyses in Kiel and pass the results on to the partners." To prevent an abuse of the project, especially through a linking of genetic and personal data by third parties, PopGen is subject to especially strict data protection guidelines. A key aspect of these guidelines is that the biological data and personal data are managed in separate databases.

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GENOMIC DNA FROM TWO-AND-A-HALF DECADES

The second large population-based project of the SMP GEMs is located at the other end of Germany. The KORA-gen project is based on a data and sample pool which has been created and maintained since the middle of the eighties in the framework of the so-called MONICA and KORA studies (MONICA stands for Monitoring of Trends and Determinants in Cardiovascular disease, KORA for Cooperative Health Research in the Region of Augsburg). In charge of the project are scientists of the GSF – National Research Center for Environment and Health. While in MONICA and KORA the focus at first was "only" on identifying risk factors for cardiovascular diseases, the research spectrum has meanwhile been expanded to include diseases such as cancer and diabetes. To date, the researchers have gathered data in four phases from more than 20,000 men and women between the ages of 25 to 74 years. In contrast to PopGen, MONICA and KORA do not specifically collect data from people with certain diseases, but rather from a cross-section of the population that is as representative as possible.

All MONICA/KORA participants undergo medical check-ups, provide information about their living habits and provide the researchers with urine and blood specimens. KORA spokesman Professor Erich Wichmann: "Thus data and bio-samples representative for the population are available to research the most varied problems; these include the genomic DNA from about 18,000 individuals." This bio-database can serve as the basis for estimating the incidence of different genotypes in the general population and enables a comparison with certain patient groups. KORA-gen was established to facilitate the use of the extensive data and sample collection for interested researchers. When they send a specific request, KORA-gen processes the needed information in coded form and carries out the relevant genotypings. As additional service KORA-gen provides expert advice and aid in questions concerning study design and statistical evaluation. Like PopGen, KORA-gen is liable to strict regulations with regard to data protection.

Christian Gieger (KORA-gen)

Max P. Baur, coordinator of the SMP GEMs, describes a vision of the impact of genetic-epidemiological studies: "In a cooperative effort of clinical scientists, molecular geneticists, and genetic epidemiologists we hope to find genetic components which are causally involved in the etiology of complex diseases." The verification of these risk factors on the population level will then be the first step towards defining individual genetic risks for these diseases. The possible consequences for prevention (lifestyle), diagnostics and treatment are manifold, but it would be unrealistic to expect quick success. Genetic medicine for complex diseases will to a large extent consist of individualized prevention.

More information

on the genetic-epidemiological projects in the NGFN is available at: www.ngfn.de
www.popgen.de
www.gsf.de/kora-gen

Uwe Radelof, Ronny Kalis and Tom-Oliver Weiss (scientists at the RZPD)

RZPD IN BERLIN – 35 MILLION DEEP-FROZEN CLONES

To do their work, gene researchers need genetic reference material – optimally in easily accessible and well-processed form. So that each single NGFN research group need not maintain its own biobank, the German Resource Center for Genome Research (RZPD) was founded in 1995 – at that time under the auspices of the German Human Genome Project (DHGP). The RZPD is the holder of two records: It is the largest service center for functional genome research in Europe and has the most comprehensive public clone collection in the world. Located in Berlin, the RZPD houses more than 1,200 genomic and cDNA libraries stored in deep freezers at -80° Celsius. Altogether there are more than 35 million clone sets from 32 different species, in particular from humans and relevant model organisms. NGFN scientists and also other research groups can access the services of the Berlin not-for-profit company. At present more than 9,000 users draw on RZPD's products and services.

The materials of the RZPD can be ordered via the Internet (www.rzpd.de). Using the search engine GenomeCube®, interested researchers can run a search to find out which materials relevant to their research are available and can be ordered. All materials are linked to internationally standardized keywords and accession numbers, so that a systematic search can be made with different search entries. Genes and gene products are the most important and largest components of GenomeCube®. For each gene of a specific species the RZPD can provide different clones, DNAs or antibodies against the respective gene product. Every year the RZPD distributes approximately 60,000 clones in this way.

RESEARCHERS CAN FOCUS ON THEIR RESEARCH

"Our main task is to provide research groups with standardized biological reference materials of high quality. In this way we support the scientific progress of the projects and contribute to the most efficient use possible of available personnel, technical and financial resources for genome research," says Dr. Johannes Maurer, scientific managing director of the

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RZPD. In other words: Gene researchers can concentrate on their actual biomedical research objectives and leave a lot of the tedious preliminary work to their RZPD colleagues. At the same time, due to the consistent high quality of RZPD's materials, the data in different experiments can be compared very well.

Usage rights to the results gained with RZPD materials or information remain with the researchers: "The RZPD raises no claims to the results and pursues no commercial interest of any kind with them," Maurer adds. "In addition, we consolidate the information gained into a database, which is freely accessible to the scientific community, and link it to additional information from public databases."

DNA chip

TECHNOLOGICAL SUPPORT

In addition to genes, DNA, etc., the Berlin resource center offers technological support to scientists in industry and research. Included are e.g. customized high-throughput technologies and automation solutions as well as extensive services for analyzing gene activity and gene regulation along with their bioinformatics analysis. For all of its services the RZPD guarantees consistent high quality. All products comply with the strict guidelines of the international standard DIN EN ISO 9001:2000 for quality management systems. Furthermore, close collaboration with German and international academic and industrial partners ensures the highest scientific and technical standard.

The RZPD is managed by a scientific director and an administrative director. In addition, an internationally renowned scientific advisory board oversees all activities. Shareholders of the RZPD are the Max Planck Society (50 percent), the Max Delbrück Center for Molecular Medicine (25 percent) and the German Cancer Research Center (25 percent).

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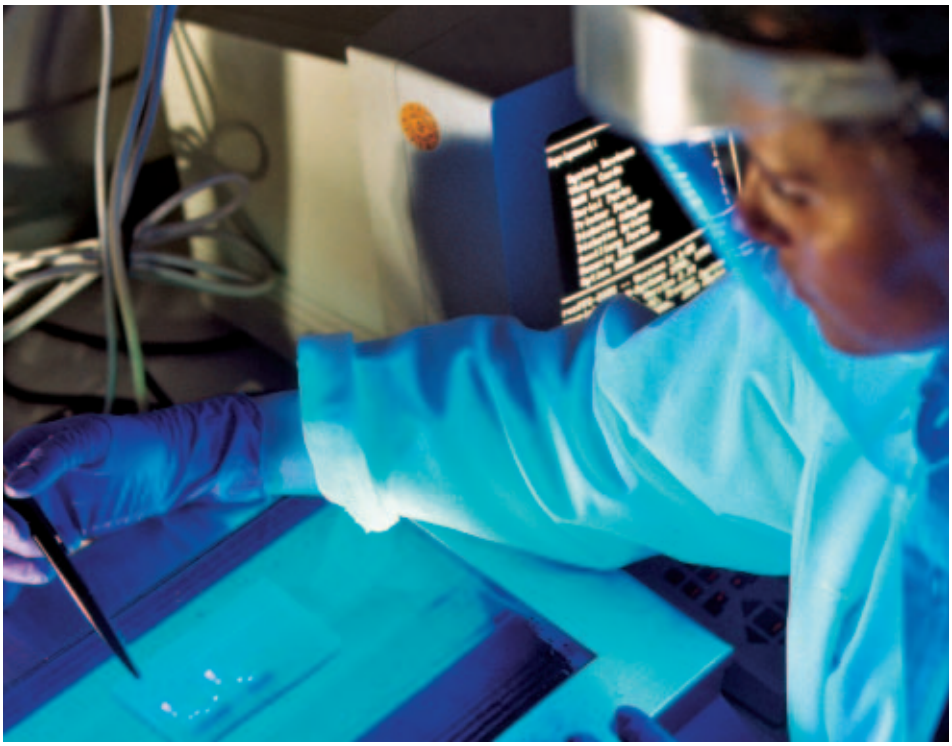
"SLEEPING BEAUTY" FINDS ITS WAY INTO GENOME RESEARCH

In the middle of the last century Barbara McClintock hypothesized for the first time the existence of "jumping genes" that change their positions in the genome. She surmised that these mobile genetic elements (transposons) are responsible for the changed color patterns that maize cobs develop in the course of generations. This theory, however, was questioned in scientific circles for a long time. It was not until about twenty years later that the existence of these transposons was definitely proven through improved molecular biological methods – and that Barbara McClintock was awarded the Nobel Prize for her discovery. Today, transposons are an important tool for genome researchers. In many model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans*, they are used to systematically decode the function of genes in large mutagenesis screens. In vertebrates, however, this technology could not be used up to now, since only "defective" transposons are known for these organisms. The sequence of these transposons is so severely mutated that they have lost their ability to "jump".



DNA gel electrophoresis

Dr. Zoltán Ivics and his team from the Max Delbrück Center for Molecular Medicine (MDC) in Berlin-Buch are now enabling the use of transposon technology in vertebrates as well. Through a comparative phylogenetic approach they have reconstructed the sequence of an active transposon from the fragments of inactive elements in the genomes of fish. "Like with the Prince's kiss in the fairy tale, we have wakened this transposon after probably 20 million years of sleep by reversing the mutations that had switched off the transposon. That is also the reason why we named it 'Sleeping Beauty'," he jokes.

However, transposons do not only find applications in basic research. In collaboration with Dr. Thomas Floss at the GSF in Neuherberg, Dr. Ivics' laboratory team is using it to trace a genetic disorder: the Williams-Beuren syndrome. This rare disease generally leads to mental retardation, but is often associated with particular abilities, e.g. with pronounced musical ability. The genetic causes of the disease are largely unknown, but a microdeletion in the gene for elastin could be detected in 95 percent of the patients. Elastin is the main component of the elastic fibers in the extracellular matrix. The expression of elastin is influenced by about 30 additional genes (so-called modifier genes) that are located in a region of 500 bp around the elastin gene. Since the deletions in the elastin gene in the mouse model do not lead to the phenotype of the disease, it is assumed that the patients have additional mutations in the modifier genes. The objective of this Explorative Project is to generate a series of mutations in the Williams-Beuren syndrome region of the mouse genome using the "Sleeping Beauty" transposon, with the hope that these mutations will recapitulate the phenotypes observed in humans. Analysis of these mutations and the affected genes will help researchers to uncover the genetic basis of this disease.

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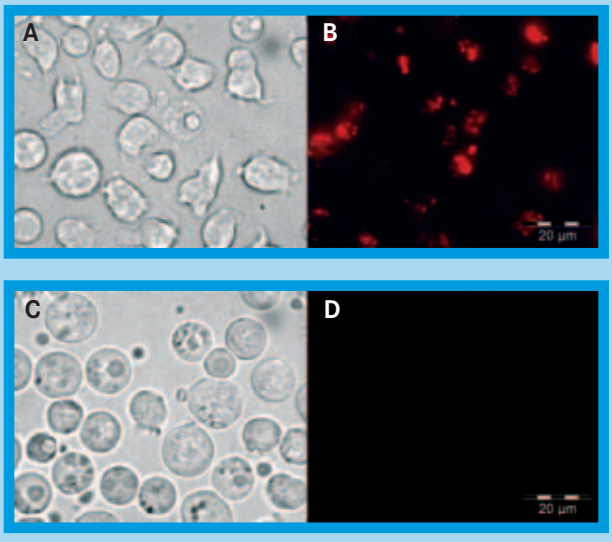
STEM CELLS – MUCH DEBATED, LITTLE UNDERSTOOD

Many areas of biomedical research place great hope in stem cells, but the molecular secret of these "miracle weapons" is still far from being understood. One reason is that many experiments are neither comparable nor reproducible. "To better compare and utilize findings from global research efforts on stem cells, we need common definitions and uniform standards. We urgently need better and more precise methods for characterizing stem cells that are rapid and reproducible," says Professor Anthony Ho, visualizing the goals of his Explorative Project "Cellular and Molecular Signatures of Human Pluripotent Stem Cells".

Stem cells possess a unique characteristic distinguishing them from other cells: They have the dual abilities to self-renew and to differentiate into different cell types. To do this they must undergo asymmetric divisions. Thus, one mother stem cell gives rise to two functionally unequal daughter cells. In previous years of research using hematopoietic stem cells as models, the research team has shown that stem cells can be separated into two groups according to the kinetics of cell division: a slow-dividing fraction (SDF) and a fast-dividing fraction (FDF). The Heidelberg researchers could match cell functions to the two fractions. The cells of the FDF are committed progenitor cells which differentiate and mature into different specialized cell types, whereas cells of the SDF, on the other hand, are destined for the purpose of self-renewal. For their experiments the scientists used stem cells derived from human bone marrow as a model. "Our aim was to pinpoint the molecular and genotypic differences between daughter cells that demonstrate the self-renewing potential versus those that are destined to differentiate and become specialists. Using a human transcriptome microarray, which represents about 95 percent of human gene sequences, we have compared the expression profiles of the two cell forms with each other and have been successful in identifying numerous genes which are up-regulated in the SDF as compared to those in the FDF," Anthony Ho explains. Furthermore, the scientists also found morphological and functional differences between the SDF and FDF: Primitive and self-renewing stem cells, found in the SDF, form numerous membrane protrusions, so-called lamellipodia, and express strongly the protein CD133 at the tip of these protrusions.



Morphology of slow- and fast-dividing hematopoietic progenitor cells. The CD34+/CD38- fraction is enriched in primitive hematopoietic stem cells. Using the fluorescent membrane dye PKH26, these cells were divided in a slow-dividing fraction (SDF; A and B) that keeps the membrane dye and a fast-dividing fraction (FDF; C and D) that dilutes the dye to their progeny. Cells in the SDF are highly polarized and demonstrate higher migratory activity and podia formation.

The interaction between stem cells and their surroundings – in particular with the cellular microenvironment – play a crucial role in determining the division behavior and subsequently the long term fate of the stem cells and their progenies. Stem cells communicate both with cells of their own type as well as with other cells that constitute the cellular microenvironment. The results of the Heidelberg researchers suggest that this communication takes place via junctions and junction complexes. Concurrently, the scientists of the group are studying how the cellular microenvironment influences the genetic profiles of stem cells. Anthony Ho emphasizes, "Decoding which factors govern the decision process self-renewal versus differentiation will not only lead to a better understanding of stem cell control and stem cell functions, but will provide a sound foundation for identifying molecules and signal transducers that play a key role in the various differentiation process. This knowledge will subsequently lead to a breakthrough for reproducibly controlling stem cell fates *in vitro* and *in vivo*."

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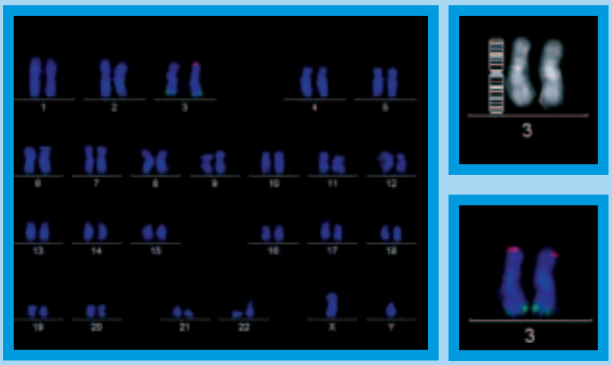
GASTROINTESTINAL DISEASES: WHAT ROLE DOES A SEROTONIN RECEPTOR PLAY?

Irritable bowel syndrome (IBS) and "nervous stomach" or non-ulcer dyspepsia (NUD) are widespread in the population, affecting approximately one in ten people. Every year these disorders lead to immense costs for the healthcare system. The patients suffer from muscle spasms in the stomach or bowel, persistent pain as well as constipation or diarrhea.



Mapping of *HTR3CDE* on chromosome 3q27

Symptoms may last for a long time and recur again and again, which is especially stressful for the affected person. Despite the high incidence, little has been known until now about the etiology of the diseases. Scientists assume that the stimulation process is disturbed between the enteric nervous system (ENS) of the gastrointestinal system and the brain (central nervous system, CNS). The serotoninergic system is also very likely involved in the etiology of the diseases. Serotonin is a messenger substance which contributes to the regulation of movements and excitability both in the brain as well as in the gastrointestinal tract. For this reason research is specifically focusing on combating gastrointestinal diseases with the serotoninergic system.

The team from the Institute for Human Genetics in Heidelberg is searching for the cause of functional gastrointestinal diseases in the serotonin type 3 receptor system (HTR3), one of the seven known serotonin receptor types. "By conducting a homology search in databases with sequences of the human genome, we found two novel *HTR3* genes: *HTR3D* and *HTR3E*. Both genes reside in close proximity and on the same chromosome," explains Dr. Beate Niesler, head of the Explorative Project "The Role of Serotonin Receptor Genes in the Etiology of Gastrointestinal Disorders". Through expression analysis, the researchers were able to determine where the two receptor genes may play a role: *HTR3D* is found in the kidneys, colon and liver, while the expression of *HTR3E* is limited to the digestive tract. "All further *HTR3* genes are expressed in numerous tissues – including the brain. The limited activity of the *HTR3E* gene in the gastrointestinal tract may be an indication that it is relevant to the pathomechanism of gastrointestinal diseases. We will now look for mutations in this gene, e.g. in irritable bowel syndrome patients. These mutations could be the cause of the gastrointestinal diseases," explains Dr. Beate Niesler. The genetic investigation should be a step forward in clarifying the role of the receptor in the etiology of the diseases. To help patients as soon as possible, the results will be collected in a public database and made available to medical and scientific institutions.

PROJECT LEADER
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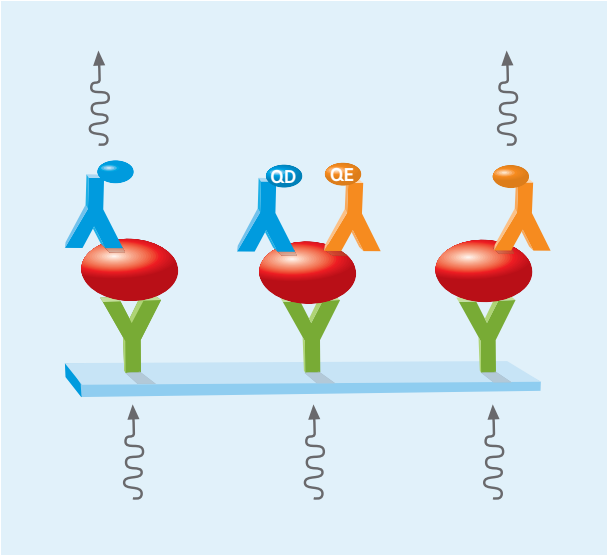
SO TEST THEREFORE, WHO BIND FOREVER ...

Nanotechnology meets protein chemistry: The project "Near Infared Nanocrystal-based Quantitative Protein Arrays" aims to detect proteins by using antibodies labeled with nanocrystals. These crystals are characterized by a wide absorption profile and a narrow emission spectrum. Due to the different emission spectra of the individual crystals, the emitted signals can be perceived clearly separate from each other. Thus, they enable simultaneous detection of many different proteins in one solution. A further advantage – the crystals show excellent photostability, enabling longer measurement times.

Proteins are often characterized by their binding to specific antibodies. High-throughput techniques for this purpose use microarray slides on which many different capture antibodies are immobilized to facilitate the simultaneous detection of multiple proteins. However, in complex mixtures like serum or cellular lysates, errors can occur: The high protein concentration in these solutions frequently causes the antibodies to bind to unrelated proteins as well. These unspecific bindings severely limit the sensitivity of the high-throughput procedures. Scientists at the German Cancer Research Center (DKFZ) in Heidelberg go for more precision: To increase the sensitivity of the procedure, they monitor the antibody-protein interactions. To this purpose, Dr. Ursula Klingmüller and her team use two antibodies: Both of them must recognize the protein and bind to it for the binding to be viewed as specific. After this a signal is emitted. But how can the binding of both antibodies be linked to signal emission? The idea of the Heidelberg scientists is based on the principle of "fluorescence quenching" – a special case of "fluorescence resonance energy transfer" (FRET). Of the two antibodies used, one is coupled to a nanocrystal, the other to a so-called "quencher". Light of a specific wave-length, which stimulates the nanocrystal, is used to detect the antibody binding. As soon as the stimulated electrons of the nanocrystal revert back to their original state, they emit energy of a different wave-length. This emitted light corresponds to the absorption spectrum of the quencher. If only one antibody binds to the protein, this energy is detected as fluorescence. However, if both antibodies have bound to the same protein and thus are in close proximity to each other, the quencher absorbs the light which the nanocrystal releases. Thus, if both antibodies bind to the protein, the emitted fluorescence is reduced or quenched. "Many diseases are based on subtle changes in protein structure or quantity. High-throughput techniques make it possible to recognize these changes rapidly and reliably. Our technique is a big step in advancing high-throughput technology. Through our use of nanocrystals and antibodies we will increase the sensitivity of detection enormously," Ursula Klingmüller explains.



Specific detection in the protein array by nanocrystals (quantum dots) in the near-infrared range. The protein of interest (red) is immobilized by a capturing antibody and detected by detection antibodies coupled to nanocrystals (QD; blue) or quencher (QE; orange).

PROJECT LEADER
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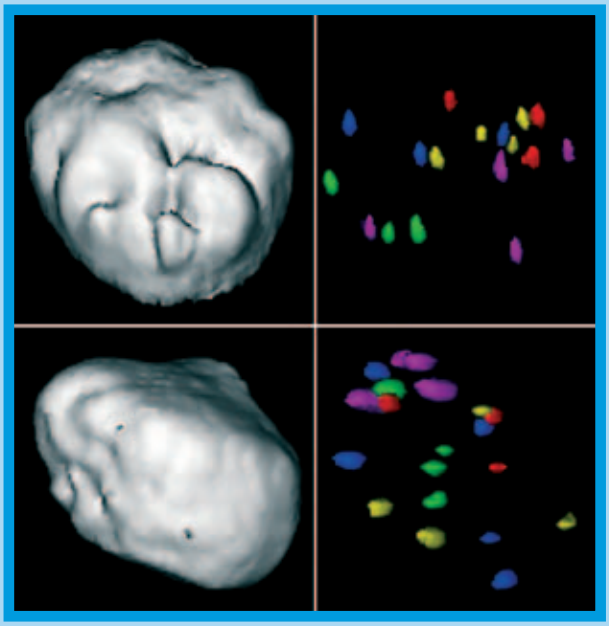
THE ARCHITECTURE OF GENES

A method for detecting precursor forms of individual cancer cells among thousands of healthy cells – for oncologists today that still seems a long way off. But when will it become a reality? "Right now we are still far away from large-scale screening at the single-cell level, but we are working on it. Moreover, we are searching for additional diagnostic markers to characterize single cells as potential cancer cells," explains Dr. Michael Speicher, head of the Explorative Project "Exploration of the 3D Genome Organization and Its Impact on Gene Expression during Tumor Development and Aging with Novel High-Throughput Multicolor Imaging Technique". Until now, a change in the number of copies in certain genome segments has been the primary indication of a cancer cell. However, it is not yet clear to what extent chromatin or position rearrangements take place prior to that. If these do take place, they could be used as markers for very early stages of tumor evolution.



Chromosomes in three-dimensional space: Using new techniques DNA can be dyed in its natural position and environment.

The aim of the Munich scientists is clear: They want to elucidate the relationship between chromatin structure and gene expression and thus identify disease- or age-related changes in the architecture of genes. To accomplish this, 3D maps of genomes from different cell systems are produced and correlated with the corresponding gene expression data. By analyzing defined chromosome regions, scientists can discover what influence the position in the genome has on the expression of a gene. High-resolution 3D maps are a prerequisite for these analyses: Their purpose is to describe the spatial arrangement of certain DNA segments down to the position of individual genes. Next, using this information, the researchers are focusing their search on disease-related changes which can be used as early diagnostic markers, e.g. for cancer. But even structural changes of the chromatin, which are caused by aging processes, could be studied in this way. In old age gene activity changes – a process about which little has been known until now. "Nevertheless, this information is important. It is the only way to distinguish between age-related and disease-related changes," explains Michael Speicher.

An important prerequisite for three-dimensional positional mapping is the simultaneous analysis of several genome segments in numerous cell nuclei. However, these comparative studies are also necessary for the later application: In order to definitely diagnose a disease, many different cells must be screened for the diagnostic markers. At present this is still a very complicated procedure that has to be repeated for each cell nucleus. It is not suited to high-throughput methods. Apart from the analysis of the 3D organization in various cell systems, a further goal of the Munich scientists is therefore to carry out these analyses in high-throughput procedures. To achieve this, they want to employ a new procedure which they have developed, based on FISH methodology (fluorescent *in situ* hybridization). For this method, probes are used which hybridize to specific regions in the genome. Using fluorochrome-labeled antibodies which bind to the probes, these regions can be visualized. Michael Speicher's team is now developing a microscope which will enable three-dimensional imaging with high spatial resolution and analysis with a high degree of automation.

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FIGHTING HEART DISEASES WITH COMBINED STRENGTHS

"It's the combination that counts!" jokes Professor Gerd Walz, head of the research project "Microarray Validation of Cardiovascular Risk Factors". His "winning combination" is his project team, which is made up of medical doctors, biologists and physicists. Together they are searching for factors leading to arteriosclerosis in terminal renal failure. Merely a short time after starting the treatment, dialysis patients often suffer from coronary heart disease, which is otherwise more likely to be found in older people.

The search begins in the Department of Internal Medicine of the University Clinic in Freiburg. By means of microarrays, Professor Gerd Walz and his colleagues are narrowing down the number of potential culprit genes. They compare cDNA samples that were taken from patients before and during a dialysis session. In addition, samples of healthy people of the same age are included in the evaluation. The researchers are especially interested in the genes that show different activity in the three sample groups. Using this method, the medical scientists hope to be able to narrow the number of potentially relevant genes down to between 1,000 and 750. At this point Professor Jens Timmer, a physicist, takes over. As data specialist he statistically determines the most promising candidates. Out of the 750 to 1,000 genes, probably about 150 candidates still remain on the short-list.

Robotics in microarray validation

The last station in the search is the Department for Bioinformatics and Molecular Genetics of the Institute for Biology in Freiburg. Here biologists on Professor Ralf Baumeister's team test the remaining candidate genes on the animal model. They switch off the genes in the nematode *Caenorhabditis elegans* – and thus obtain information about the functions of the gene products. "Our threefold approach enables us to close the information gaps between the high-throughput screenings, the genetic backgrounds and the diseases – a model that can set a precedent," Gerd Walz says delightedly.

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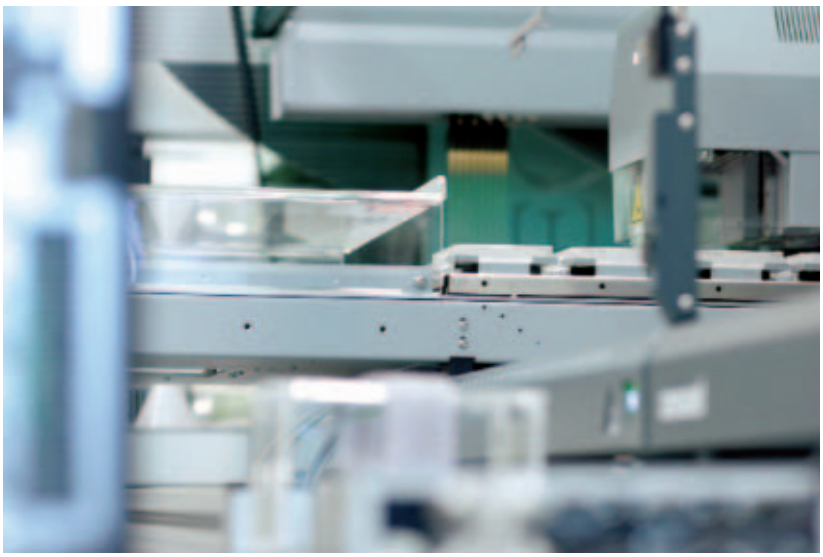
METHUSELAH'S HEIRS – WHERE IS THE GENETIC SECRET?

Today more and more people attain a "biblical" age while often still leading healthy and active lives. Epidemiological studies indicate that the phenomenon of healthy or successful aging in humans is inherited to about 30 percent. The Explorative Project (EP) "Genetic Etiology of Human Longevity" aims to characterize the genetic factors which contribute to a long and healthy life as well as to identify molecular pathways that are associated with the physiology of aging and/or age-related diseases. This EP is a multicenter collaboration in which the following institutes participate: Institute for Clinical Molecular Biology, University Hospital Schleswig-Holstein, Kiel (Professor Stefan Schreiber); Department of Dermatology, Charité University Medical Center Berlin (Professor Christos C. Zouboulis); Max Planck Institute for Molecular Genetics, Berlin (Professor Hans Lehrach).



High-throughput genotyping

Already during the NGFN's first funding phase, scientists at the Institute for Clinical Molecular Biology in Kiel collected DNA samples from more than 2,500 long-lived (95 years and older) and younger control individuals (60–75 years) from across Germany. Of these, 430 samples are from centenarians. The DNA collection in Kiel is thus one of the largest biodatabases of its kind worldwide. The first clues about genes that can extend life span were gained from studies on various model organisms such as flies, nematodes and mice. The scientists in Kiel are currently conducting a large-scale study to investigate to what extent these "longevity genes" also play a role in humans. In their search the researchers make use of single nucleotide polymorphisms (SNPs), the most common type of genetic variation in the human genome. Thousands of SNPs are analyzed in several hundred functional candidates, which in model organisms are known to be relevant to longevity and aging (e.g. energy metabolism, anti-oxidation or apoptosis). The SNP data of the long-lived individuals are then compared to those of the younger control group. The idea is to find out which of the tested genetic variants occurs more or less frequently in the elderly than in the younger population. The so-identified SNPs indicate genes that may be involved in longevity or aging in humans.

In addition, the researchers of the EP are also interested in the metabolic pathways of aging. Using DNA arrays, the teams in Berlin are comparing the expression patterns of skin cells of different origins: skin cells from human cell cultures, from human tissue samples of twenty-year-olds and sixty-year-olds, and from tissue samples of long-lived mice. Differences in the expression profiles will allow conclusions about the molecular pathways of aging. The scientists are searching for genes that are more or less highly expressed in tissues of younger people than in those of elderly individuals. Subsequently, using the technology of siRNA, the Berlin scientists plan to identify the functional role of these genes on cell life and behavior and compare their findings with the Kiel data.

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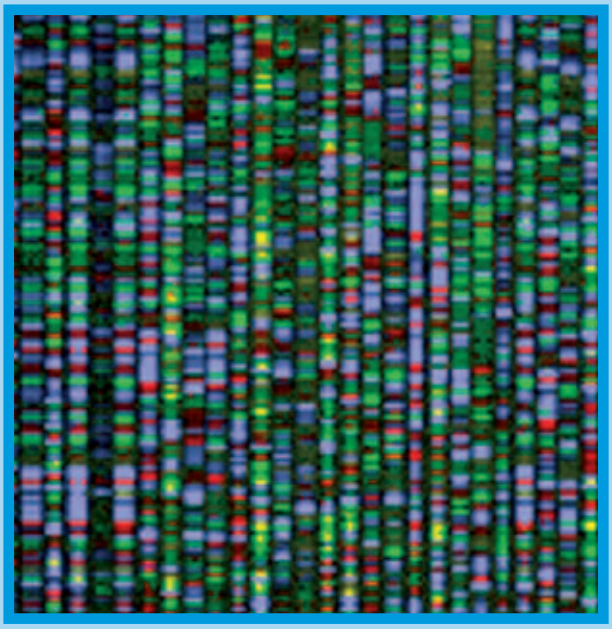
HEALTHY DESPITE HIV

In some people an effective protection against AIDS (Acquired Immune Deficiency Syndrome) is encoded in their genes. Even more than ten years after the infection with HIV (Human Immunodeficiency Virus), the disease does not break out in these patients. Only extremely small quantities of the virus can be found in their blood – a sign that it hardly replicates at all. "This knowledge should make us hope. We must find out what factors are holding the virus in check in these people. Perhaps that is an important step in the struggle against AIDS", says Dr. Matthias Platzer, head of the Explorative Project "Genomic Variability of Host Factors in the AIDS Macaque Model – Role in Resistance to and Disease Course of Viral Infections in Humans and Non Human Primates" at the Leibniz Institute for Age Research – Fritz Lipmann Institute (FLI) in Jena.

An interdisciplinary team is now searching for the crucial genes in the rhesus monkey. The team's members include the immunologist and virologist Dr. Ulrike Sauermann and Professor Gerhard Hunsmann of the German Primate Center in Göttingen, the Kiel mathematician Professor Michael Krawczak, the genetics professor Peter Nürnberg of the University of Cologne and the microbiologist Dr. Roman A. Siddiqui, who also works at the FLI in Jena. In Rhesus monkeys the SI-Virus (Simian Immunodeficiency Virus) elicits an immune deficiency which is very similar to the human HIV infection. Since some of these animals do not develop an AIDS-like disease after infection, they are an ideal model for researching resistance mechanisms. "The monkey model gives us the advantage of having a great deal more data about the infection, for instance the kind and time of the transmission, the quantity and type of the infectious virus and the complete course of the disease. Above all, we know the personal and familial medical history of the infected primates. Such data are very valuable for systematic studies and are never available for humans," Matthias Platzer adds.



DNA sequencing

The NGFN researchers are searching through the entire genome of the infected primates. They are looking for genetic markers which only occur in resistant animals. Using these markers they can limit the areas in the genome that make survival possible after such an infection. Sequencing these DNA segments should then identify the particular factors in the genome, which protect resistant monkeys from falling ill. The results of the search will be entered into a database made available by the genome networks Infection and Inflammation and Environmental Diseases. The cooperation with the competence network HIV/AIDS in turn ensures that the results gained in the animal model are quickly incorporated into human AIDS research.

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ALTERNATIVE mRNA SPLICING: MORE INFORMATION FROM FEW GENES

How can a complex organism like the human being function with only about 30,000 genes? After all, even the simple nematode worm *Caenorhabditis elegans* has about 19,000 genes. One answer is alternative mRNA splicing. Probably more than half of all human proteins are produced via this mechanism. But what lies behind this?

Sample preparation

Alternative splicing can best be explained by stating what it is not: constitutive splicing. In constitutive splicing the coding segments (exons) – after removal of the non-coding sequences (introns) – are joined in the sequence in which they also occur in the DNA. From a pre-mRNA always the same mRNA is produced. Due to alternative splicing the sequence of the mRNA is varied: individual exons can be shortened or lengthened, entire exons can be skipped. Through these processes many mRNA variants and thus also many different proteins are generated from one single gene. Often these processes are regulated in a tissue-specific and development-specific manner.

The Explorative Project "Analyzing Global Regulators of Alternative Splicing in the Human System: A Combined RNAi and Microarray Approach" deals with the underlying mechanisms of alternative splicing. "Until now little has been known about which proteins regulate these processes and what their target sequences look like in the RNA. Our goal is to systematically identify the complete networks of splicing regulator proteins and their target genes in the human genome," says project leader Professor Albrecht Bindereif. A new methodical approach is to help with this: Via RNA interference putative splicing regulators will be targeted individually and switched off. By using microarrays it can then be analyzed what effects switching off the regulator has on the splicing pattern of certain target genes. For this purpose splicing-sensitive microarrays are to be used that simultaneously allow the tracking of the alternative splicing pattern of a great number of candidate genes.

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CANCER VACCINATION: ARE WE MAKING PROGRESS?

The immune system is our best weapon in the fight against diverse pathogens. Utilizing this fact, scientists are attempting to enlist the immune system to combat one of the greatest scourges of humanity: cancer. Vaccination against cancer using T lymphocytes (T cells) to activate the immune defense is a very promising research approach. T cells have highly specific receptors for the identification of their antigens. The binding to the receptor triggers an immune response which destroys the "agitator" (see box). However, a successful vaccination implies the presence of tumor-associated antigens that the immune system can target. Characterizing these antigens is therefore one of the central tasks of today's cancer research. But where do we find these antigens? Good candidates are proteins frequently produced in tumor cells but rarely or never present in healthy cells. In general, cancer scientists search for these proteins by means of the cells' mRNA quantity. With high-throughput screening methods such as DNA arrays, this is effective and cost-efficient. Ultimately, however, it does not play a decisive role how much protein is present in the cell. More important for a successful immune response is the frequency with which a protein is presented on the surface, for here is where the contact with the T cells occurs. Until recently, it was not possible to determine the quantity of MHC-bound (see box) protein fragments presented on the cell surface. Therefore, it was not clear whether proteins with increased mRNA quantity in tumor cells are also presented with higher frequency on the surface of the cell. That, however, is a prerequisite for proteins to be used as effective antigens in the fight against cancer. The Explorative Project headed by Professor Hans-Georg Rammensee has solved this problem: Using mass spectrometry, scientists can focus their investigations on how frequently an MHC-bound protein fragment appears on the cell surface. "First we are checking the methods established up to now. If we can prove that proteins with an elevated mRNA quantity are also present in increased quantity on the cell surface, we can go ahead and continue working with these methods. However, if this is not the case, this would cast much doubt on these previously used approaches. Our mass spectrometry analyses would then be the only reliable possibility to look for tumor-specific antigens," Professor Rammensee explains.

HOW DOES A T LYMPHOCYTE IDENTIFY "ITS" ANTIGEN?

The cell places small fragments of all proteins which are present in the interior of the cell in a "gallery" onto the cell surface. The cell's own mechanism ensures that all proteins present in the cell interior are continually presented on the surface. A constant circulation of degradation and production of proteins takes place in the cell. During the degradation process protein fragments are created, of which small pieces are bound by specialized receptors (MHC = Major Histocompatibility Complex) and transported to the surface. The T lymphocytes can differentiate whether these fragments are either known and normal, or foreign and abnormal, respectively. If they identify the protein as foreign or pathogenic, they kill the cell and stimulate the production of antibodies against the antigen.

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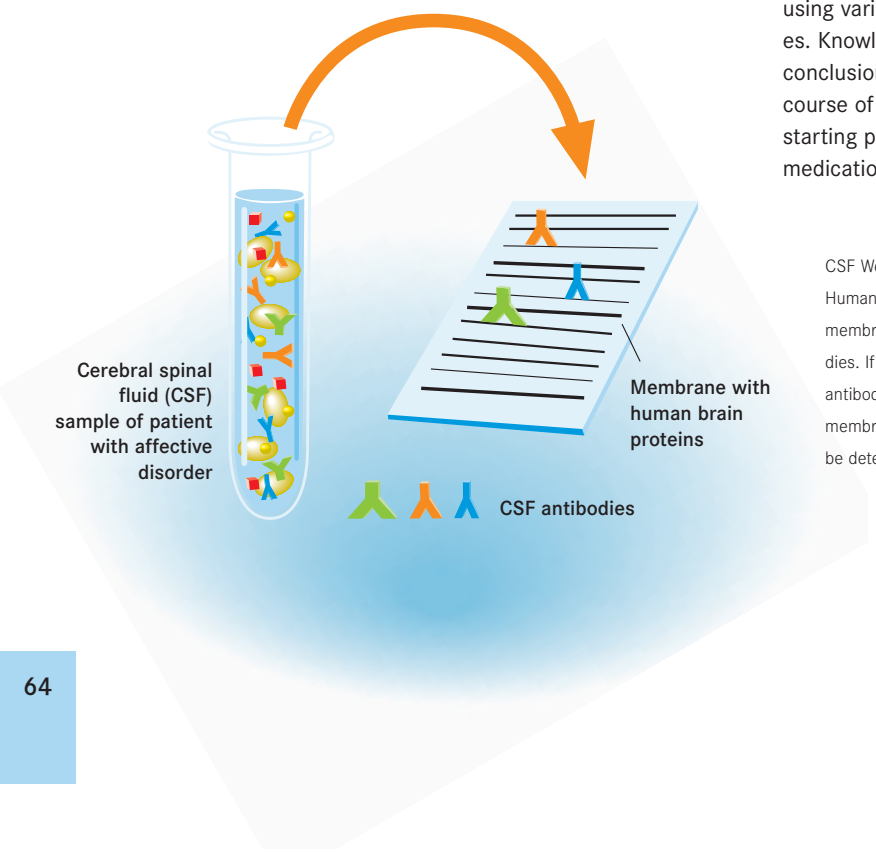
DEPRESSION – A WIDESPREAD ILLNESS

Depression and anxiety disorders are among the greatest medical and healthcare challenges of our time. In the industrial countries twelve to 20 percent of the population will fall ill with a depression at least once in their lives. The basic mood of the afflicted persons is sad, their drive is diminished, and they lose interest in their social surroundings. They feel helpless and are despairing. Forty to eighty percent of the affected persons even think of committing suicide. One major problem is that depression is often not recognized and is diagnosed much too late.

The Explorative Project "Identification of Autoantigens as Candidate Markers for Affective Disorders", headed by Professor Christoph W. Turck, wants to contribute to an improved diagnosis of affective disorders. His team is searching for characteristic markers which only occur in combination with affective disorders by taking advantage of the immense precision of the human immune system. The scientists are searching for autoantibodies which target the proteins of the brain tissue in the CSF of the patients. CSF is an abbreviation for cerebrospinal fluid, which circulates through the ventricles of the brain and the cavities of the spinal cord. "The CSF provides a reflection of the healthy or sick brain," Christoph Turck explains, "which we were able to show in large-scale analyses of CSF proteins."

In their experiments the scientists were also able to show that antibodies from the CSF recognize proteins of the human brain. In previous experiments they established an immunoassay that enabled this determination. They separated the proteins of the human brain tissue electrophoretically and transferred them to a membrane. They incubated this membrane with CSF samples of patients with affective disorders. Here they discovered first interactions between autoantibodies and brain proteins. Currently, in a larger scale experiment, scientists are systematically screening the CSF of patients with affective disorders to detect other autoantibodies. The brain proteins identified by means of the autoantibodies are the sought-after diagnostic markers for affective disorders. Preventive measures can then be taken in the clinic as treatment.

Moreover, these proteins could also provide the basis for further experiments: Professor Turck and the members of his working group want to decode the functions of the proteins using various molecular biological and biochemical approaches. Knowledge about the protein functions could then lead to conclusions about their significance for the pathogenesis and course of affective disorders. That could be an important starting point for pharmaceutical research to develop new medications for affective disorders.



CSF Western blot analysis:
Human brain proteins immobilized on a membrane are probed with CSF antibodies. If the CSF contains a brain-specific antibody it will bind to its antigen on the membrane and generate a signal that can be detected by an X-ray film.

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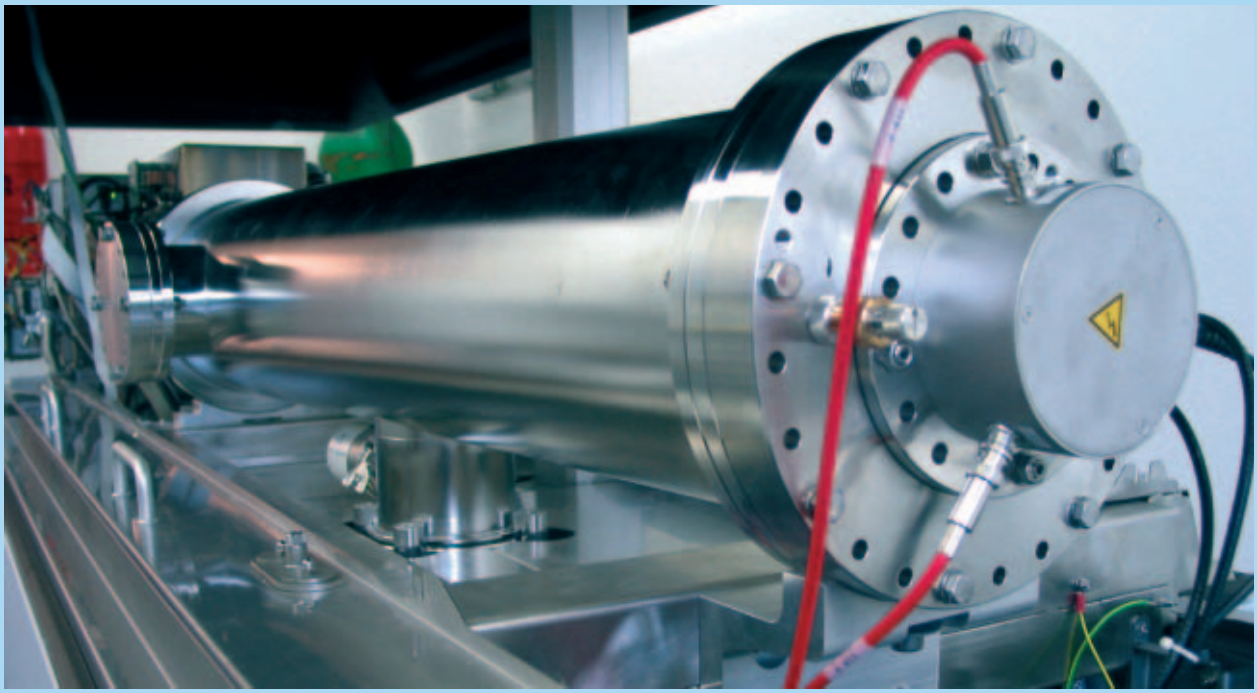
NEWS FROM MASS SPECTROMETRY

Biological macromolecules are often large and very complex. This is why for a long time it was not possible to use mass spectrometric analyses in biology, although in analytical chemistry, elements and small molecules – or their fragments – were routinely determined with this method. It was not until the development of gentle ionization methods (MALDI, ESI) in the middle of the 1980s that mass spectrometry came into use in biological and biochemical laboratories.



Mass spectrometer

This was a milestone: From then on biological macromolecules could be transferred intact into the gas phase and could be ionized for analysis. In 2002 the Nobel Prize in Chemistry was awarded for these achievements, illustrating the current significance of mass spectrometry. Since then the technology has been developed even further, especially with regard to the achievable sensitivity. At present even the smallest quantities of less than one femtogram (10^{-15} gram) can be detected. Advancements in mass spectrometry are being pursued worldwide: Professor Bernhard Spengler and his team from the University of Giessen are working on a mass spectrometry method which can image the composition and the spatial distribution of biochemical compounds. The projected methodology is an extension of the SMALDI technology (scanning microprobe matrix-assisted laser desorption ionization) developed by Spengler and his team in recent years, and it has unique analytical features. It involves a high-power laser that scans e.g. a cell surface; the laser-ablated molecules are subsequently analyzed using mass spectrometry. Finally, a software program evaluates the data with respect to composition as well as to the local distribution of the individual substances. In this way an image is created that e.g. shows the concentration gradient of a substance on the cell surface. "In the future we plan to use an infrared laser instead of a UV laser for this system. That would have several advantages: Infrared light penetrates up to one micrometer deep into the specimens – while UV light reaches layers that are maximally 100 nanometers deep. Infrared lasers would enable us, for instance, to study even deeper regions of a tissue," Bernhard Spengler explains. But this is not the only advantage: For many biological substances, such as carbohydrates and lipids, infrared light is superior to ultraviolet light with respect to accessible mass range. Moreover, with an infrared laser the laborious task of sample preparation would be simpler. While for an analysis with UV light the sample has to be embedded in a special matrix, for an infrared laser it is in principle sufficient to deep-freeze it. He adds, "Until this is accomplished we still have some work to do. It is important to note that there is not yet any infrared laser that can ensure such a high resolution. Likewise, a system has yet to be developed to evaluate these data."

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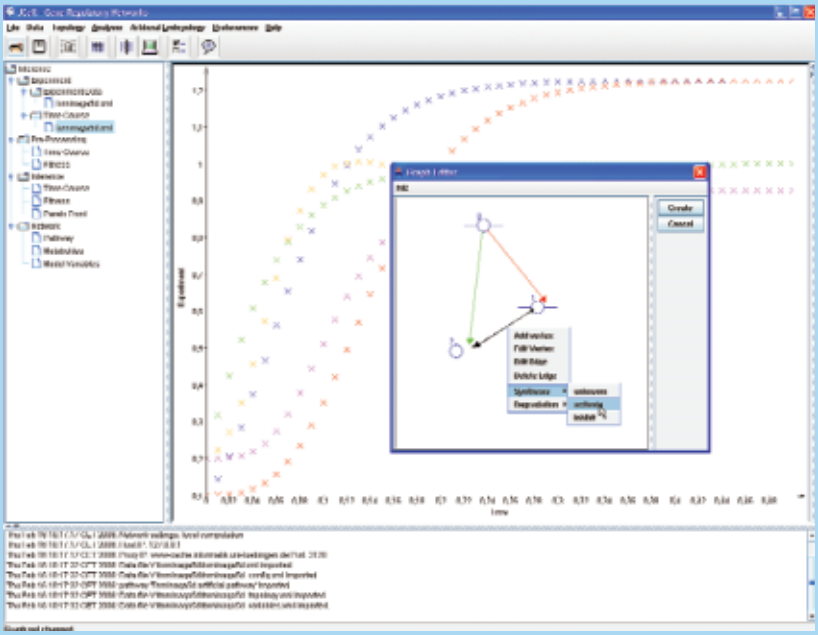
INFERENCE MODELS FOR GENE REGULATION

The goal of the bioinformatics team under the direction of Professor Andreas Zell is to develop inference models for gene regulation networks. "Based on the vast quantity of data available from microarrays, we reconstruct parts of the regulatory network of the genome," Andreas Zell explains. "For example, if we have information on time-series gene expression – which genes are active when – we can conclude from that how they interact with each other." The team from the Center for Bioinformatics at the University of Tübingen has developed a software program that enables these conclusions: From the experimental data JCell directly infers regulatory relationships among the genes studied.



Screenshot of the software framework JCell

Until now microarrays were mainly used to compare the activity of genes independently of each other, e.g. to analyze gene expression in healthy and diseased tissue. However, mutations in a single gene may often lead to changes in many different cell functions. JCell can help scientists identify regulatory relationships – and thus glean more information from the data. "With the current version of JCell, gene researchers can reconstruct small genetic networks, at least if they have sufficient experimental data. And we are continually optimizing the system to improve the inference algorithm design of natural interactions," explains Christian Spieth, member of the Explorative Project "Inferring Genetic Interactions from Gene Expression Data". JCell also accesses public databases. "This is how we guarantee that the most up-to-date expert knowledge is included in the analysis of gene regulation processes," he adds. In the future, it is planned that JCell will also simulate complex biological processes and their disease-related variants. The program could then make a considerable contribution to the understanding of complex diseases such as infections or cancer and open up new therapy approaches.

JCell is currently one of the most effective analysis tools for reconstructing and simulating the dynamic systems of gene regulation. For this achievement the project won third prize in the 2005 doIT Software Award Competition, a research initiative of the state of Baden-Württemberg.

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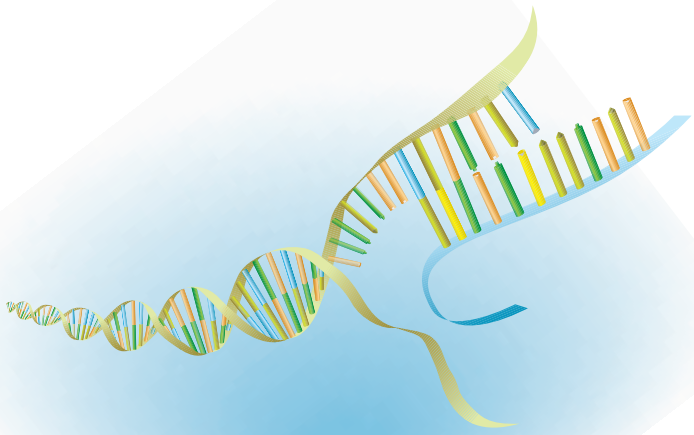
IS OUR GENOME WASTEFUL?

In recent years it has become ever more evident that our genome produces several thousand transcripts which do not code for proteins. Does that mean that our genome is wasteful? Or do these mainly small RNAs have tasks in the cells that are not yet known? "In my opinion many of these RNAs perform other functions, for example, they regulate gene activity. The emphasis is on many, not all. Today we still do not know whether most of them have a job to do in the cells or not," explains Professor Jürgen Brosius, head of the Explorative Project "Exploring the World of Non-Messenger RNAs: RNomics Meets Proteomics". The Münster scientists want to find answers to these questions. With different high-throughput procedures they are searching specifically for non-protein-coding RNAs in mammals. "When such RNAs are found, we try to find out whether they take over functions in the cells. And next, if this is the case, we want to find out what they do there," Jürgen Brosius says, describing the objective of his project.

> *"Nor ought we to marvel if all the contrivances in nature be not, as far as we can judge, absolutely perfect; and if some of them be abhorrent to our ideas of fitness. We need not marvel at ... the astonishing waste of pollen by our fir trees."*
>
> Charles Darwin

One thing is already considered certain. These new RNAs enable genetic diversity because they represent the raw material for new sequence modules in the evolution of genomes. These develop through reverse transcription of RNAs or through random integration into the genome. Depending on where these modules land, the effect can be disadvantageous, neutral or even beneficial. For instance, this is how new exons are formed out of non-coding sequences – an important mechanism of evolution. Often, splice variants of already established genes are generated. The old and new forms of the gene then exist parallel in the genome. Thus, the new form can be tested while the old form continues to be in use. If the new splice variant does not offer the organism any advantage, it disappears in the course of time. The researchers of the Institute for Experimental Pathology are also pursuing this approach. They have investigated 153 exons in the genome in whose development Alu elements were probably involved. Alu elements belong to the family of short interspersed elements (SINE) and are specific to primates. Some of them are so similar to splice sites that they induce alternative splicing. Other Alu elements can be transformed into a splice sequence through a simple nucleotide exchange. The research group led by Jürgen Brosius compared four selected examples more exactly between the species of primates. Result: They were able to reconstruct the evolutionary steps that occurred in the various species and thus could decode the significance of the elements for evolution. According to these findings, after integration into the genome the Alu elements either induced the alternative splicing directly or slumbered unrecognized for millions of years until they became active.



PROJECT LEADER
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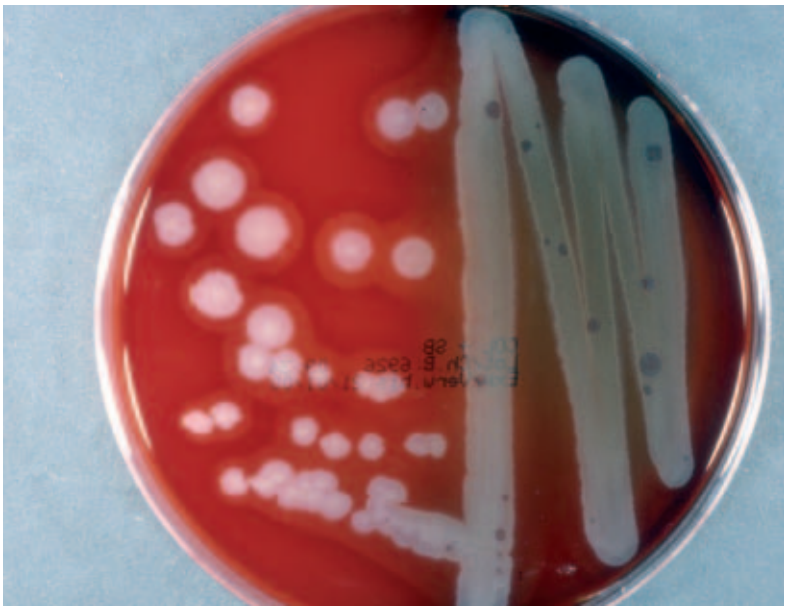
A "HOSPITAL BUG" BECOMES PREDICTABLE

Contact with *Pseudomonas aeruginosa* cannot be avoided, because these resistant bacteria are found nearly everywhere in our environment. In most cases, however, the human immune system prevents disease caused by this typical moist-site, waterborne pathogen. By contrast, immunocompromised patients often have no protection against infection with P*seudomonas aeruginosa*. In cystic fibrosis (CF) patients, for example, the predominant cause of death is pneumonia caused by *Pseudomonas aeruginosa*. Cancer patients and burn victims are also particularly susceptible to this pathogen, since *Pseudomonas aeruginosa* can trigger wound infections, sepsis and cardiovascular diseases.



Culture of *Pseudomonas aeruginosa*

In CF patients *Pseudomonas aeruginosa* frequently attacks the respiratory tract and colonizes the thick mucus which is typical of the disease. There the bacteria form a biofilm which enables them to evade the body's own defense mechanism. Even many antibiotics cannot penetrate this protective envelope and are therefore ineffective against the pathogen. Moreover, Pseudomonas also generates enzymes which directly inactivate the antibiotic. Once the bacteria have colonized the lung, they cause inflammation which leads to progressive lung damage of the patients and is the major cause for morbidity and mortality in CF. Professor Dieter Jahn and his team at the Institute of Microbiology at the Technical University Braunschweig are attempting to elucidate the exact processes which lead to the formation of the biofilm and to resistance against antibiotics. To achieve this, they want to thoroughly analyze the metabolic products of *Pseudomonas aeruginosa*. "What we need is a combination of very advanced high-throughput procedures and sophisticated bioinformatics to attain this objective. After all, we want to investigate all metabolic products of this bacterium during an infection of the lungs. But we first have to develop the appropriate high-throughput procedures," Dieter Jahn explains. The findings from this approach will first be compared with existing data from proteome and genome analyses in order to draw possible conclusions about the development of metabolites. Two established databases are available for this: BRENDA (BRaunschweig ENzyme DAtabase) and PRODORIC (PROcariotIC Database Of gene Regulation). BRENDA is managed by Professor Dietmar Schomburg and his colleagues at the Cologne Institute for Biochemistry. The database contains the largest collection of data anywhere in the world on enzymes and metabolic pathways. PRODORIC, on the other hand, is managed by the Braunschweig scientists and is the largest database worldwide on regulatory networks of prokaryotes. In particular, the PRODORIC database organizes information on pathogens. "Our objective is to develop a model for *Pseudomonas aeruginosa* – a model that will represent all gene-regulatory and metabolic pathways of the pathogen," says Dieter Jahn, looking to the future.

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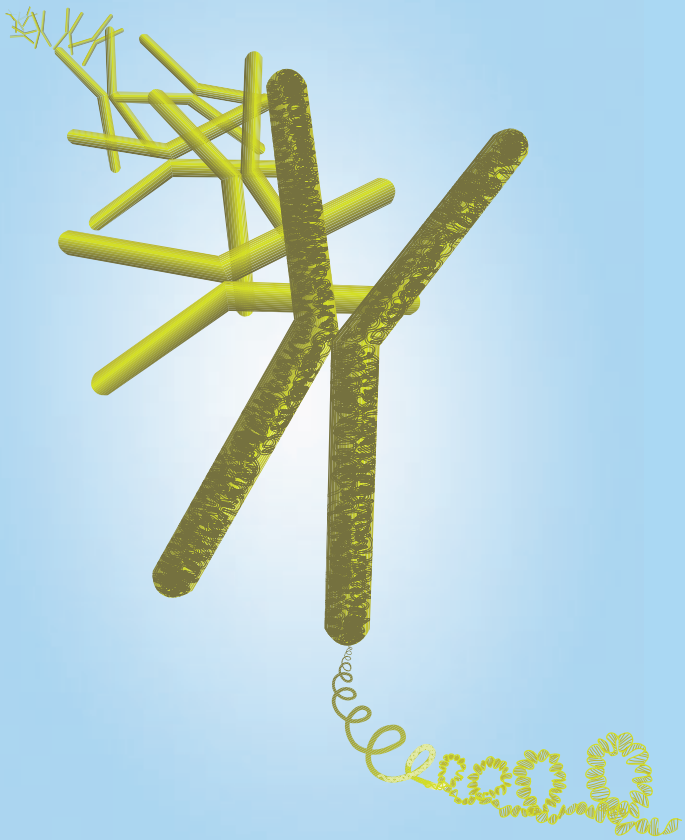
FROM GENOME TO ORGANISM – THE WHERE AND WHEN OF GENE EXPRESSION

A textbook "Molecular Biology of the Cell" (Bruce Alberts et al., 3rd edition) introduces the chapter about gene expression as follows: "An organism's DNA sequences encode all of the RNA and protein molecules that are available to construct its cells. Yet a complete description of the DNA sequence of a genome – be it the few million nucleotides of a bacterium or the three billion nucleotides of a human – would provide relatively little understanding of the organism itself. It has been said that the genome represents a complete 'dictionary' for an organism, containing all of the 'words' for its construction. But we can no more reconstruct the logic of an organism from such a dictionary than we can reconstruct a play by Shakespeare from a dictionary of English words." In both cases the problem is the same: Where and when is each word used, or rather, which gene is expressed?

Gene expression in the cell is strictly controlled. Transcription plays a key role in this. Many regulatory mechanisms adhere to this first step of gene expression and thus enable the different developmental and differentiation processes of the cells. Transcriptional control is enabled via protein-DNA interactions. For scientists to understand these control mechanisms, they must trace these interactions and analyze them. This generally takes place via chromatin immunoprecipitation (ChIP) technology: First the DNA of a cell is cross-linked with regulatory proteins. Via antibodies that specifically bind to the cross-linked proteins, the scientists purify the DNA protein complexes. The thus-extracted DNA sequences are copied using PCR and subsequently analyzed. In model organisms such as yeast, DNA microarrays representing the entire genome of the organism have been used in recent years.

The Explorative Project "Synthetic Antibodies for ChIP-chip in Human Cells" is searching for the target sequences of the regulatory proteins in humans. "In the human genome the use of microarrays for ChIP technologies has not been possible until just recently. The reason for this is that the human genome is considerably larger and more complex than that of yeast, for instance. The development of comparable methods presents an enormous technical challenge for science," explains Professor Michael Meisterernst, who heads the Explorative Project. Using synthetic antibodies, he and his team aim to develop ChIP-chip technology further for the human system. These synthetic immunoglobulins bind highly specifically to certain regions in their target protein. In contrast to their natural counterparts, synthetic antibodies can also target short signal peptides which do not occur naturally. This enables researchers to express numerous different proteins in the cells which all contain the same signal peptide and which therefore can be detected by the same antibody. Alternatively to this, the Munich scientists are developing antibodies that directly recognize a natural protein. Since highly specific antibodies which can be used for ChIP-chip technology are relatively rare, the genome researchers are



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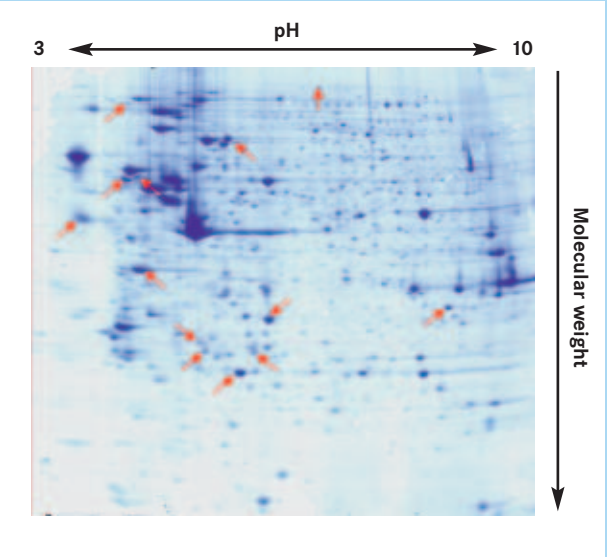
INNOVATIVE APPROACHES FOR TREATING RENAL CANCER

Renal cancer is the tenth most common cancer in developed countries, accounting for approximately three percent of all diagnosed malignancies with up to 95,000 deaths per year worldwide. More than 75 percent of renal cancer cases are classified as renal cell carcinomas (RCC). At presentation less than 40 percent of the patients have tumors confined to the kidney – they have developed metastatic disease and have a five-year survival rate ranging from 16 to 32 percent. For localized disease, nephrectomy is the main treatment,



Two-dimensional polyacrylamid gel: Differences in the protein expression pattern of treated versus untreated RCC cells. Exclusively expressed or significantly upregulated proteins in the treated variants are marked by red arrows.

whereas the efficacy of other treatment modalities, such as radiotherapy and/or chemotherapy, is very limited. No information is available regarding valuable prognostic and diagnostic as well as broadly applicable therapeutic markers for RCC. Currently, the performance status, tumor stage and grade are the most useful clinical prognostic indicators in patients with RCC. Although RCC is – analogous to melanoma – a relatively immunogenic tumor and biological therapies appear to be successful, the number of RCC-specific antigens is still very limited and the subjective response rate is less than 20 percent. Therefore the identification of novel tumor-associated antigens (TAAs) as well as novel concepts for the treatment of this disease are urgently needed.

The Explorative Project "Combining Proteome-based Technologies with Epigenetics Using Renal Cell Carcinoma (RCC) as a Model: Identification of RCC Specific Cancer Antigens for Vaccine and Antibody Therapies" is taking on this task. Epigenetic events, such as deregulated methylation of CpG dinucleotides and aberrant histone acetylation are frequently occurring modifications at the DNA level. If such events occur close to and/or within open reading frames (ORF), this will likely lead to a reduced or even fully silenced gene expression and thus may have an impact on diverse protein functions. Abnormalities in DNA methylation and histone acetylation have been shown to play an important role in both tumor development and progression, thereby impairing the immunogenic potential of cancer. It has recently been shown that DNA hypermethylation and/or histone deacetylation contribute to the lack or deficient expression of components involved in tumor recognition like molecules of the antigen processing and presentation machinery and/or of the costimulatory pathways.

Thus, the scientists treat a panel of primary tumor-derived cell lines, representing both malignant RCC subtypes such as (i) clear cell, (ii) chromophobic and chromophilic RCC as well as the benign oncocytoma with demethylating or deacetylating agents to overcome given tumor-related epigenetic blocks. In a systematic effort to identify such cancer-related epigenetic changes, the research team will employ two-dimensional gel electrophoresis followed by mass spectrometry and PROTEOMEX, a method which combines proteome analysis with immunoblotting using sera from healthy donors and RCC patients to analyze pattern changes in the protein expression profiles under experimentally designed epigenetic conditions and thereby assess their impact on the immunogenicity of the treated tumor cells. "In our previous experiments we succeeded in defining differentially expressed proteins between primary tumors and healthy renal epithelia," Professor Barbara Seliger explains. Now the scientists are attempting to characterize the mechanisms by which tumor cells install epigenetic pattern changes. The analysis of the expression profiles of untreated versus treated tumor cells might not only allow to define novel biomarkers, but also might lead to the development of novel immunotherapeutic relevant concepts linked to the pursued reversion of epigenetic phenomena.

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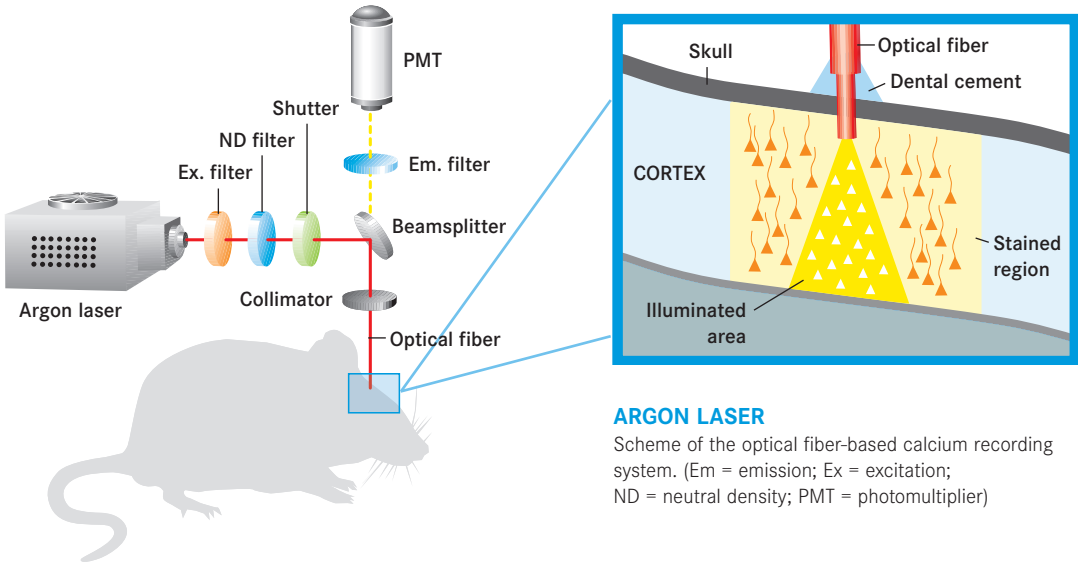
LOOKING INTO THE CENTER – A NEW TECHNOLOGY ENABLES IN VIVO STUDIES IN THE BRAIN

The brain awakens the curiosity of scientists and non-scientists alike. For years, Professor Arthur Konnerth and his colleagues at the Institute for Neuroscience of the Technical University in Munich have been studying the function of the central nervous system. The researchers focus on the development and the plasticity of neural networks in the brains of animal models, mostly mice and rats. Their results show that during brain development, large groups of nerve cells are active simultaneously. Characteristic activity patterns produce prominent changes in the calcium concentration in virtually all neurons. These changes in concentration spread in a wave-like fashion through the cortex and have a key function during the early phase of brain development. "However, *in vivo* – that is, in the intact brain – we were not able to prove the presence of these calcium waves because we lacked the proper technology. Until recently we could only perform *in vivo* experiments on anesthetized test animals. We now know, however, that the anesthesia of the mice prevents the calcium waves," Professor Konnerth explains.

The NGFN scientists thus had no other choice – they had to develop a new *in vivo* technology. In the framework of the Explorative Project "Brain Endoscopy for the Functional Analysis of Neuronal Networks *in vivo*" a suitable method has now been established. "To monitor Ca^{2+} levels in awake behaving mice, we have developed an optical fiber-based recording system consisting of a chronically implanted fiber used both for excitation of the dye and for detection of the emitted fluorescence. This single-fiber endoscope allows recording of Ca^{2+} signals from any brain region in anesthetized as well as awake behaving animals. Therefore, it offers a unique opportunity for studying brain function and for detecting the action of drugs without the influence of anesthetics," Dr. Helmuth Adelsberger comments. As a first step, the desired brain regions have to be stained with calcium-sensitive fluorescent indicator dyes. Upon binding Ca^{2+} ions, such dyes change the spectral characteristics of the emitted light. The emitted light is monitored with sensitive detectors and translated into intracellular alterations of the calcium concentration. "Further advantages of this system in contrast to other methods used for these purposes are the possibility to implant the optical fibers in deeper layers of the brain. By using several fibers simultaneously, the interaction of different brain regions can be studied in real time," Dr. Helmuth Adelsberger adds. "With this new system we can extend our studies on brain development and address open questions. Utilizing the new approach, we recently found that anesthesia blocks the intracellular calcium oscillations. In non-anesthetized mice we were able to clearly identify and characterize the calcium oscillations. We have now started to study altered calcium signaling in mutant mouse disease models with the single-fiber endoscope," says Professor Konnerth.

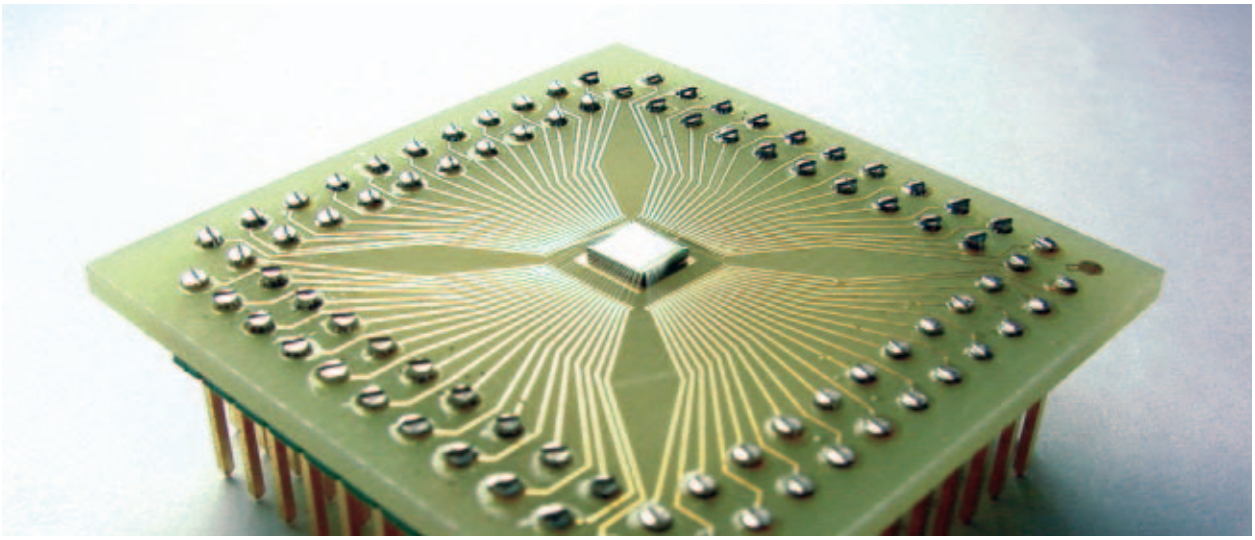


ARGON LASER
Scheme of the optical fiber-based calcium recording system. (Em = emission; Ex = excitation; ND = neutral density; PMT = photomultiplier)

A DRAGNET OPERATION TO FIND BINDING PARTNERS

While genome research today has highly complex DNA arrays at its disposal, the spot density of peptide arrays is comparatively low: Between 10,000 and 50,000 peptides can be synthesized on a chip of 20 x 20 cm using current technology. One reason for this is the more complex binding chemistry of the peptides, which makes their production more difficult. But also the greater number of components in comparison to DNA play a role. For the synthesis of peptides 20 different amino acids have to be combined – instead of only four nucleotides in the production of DNA arrays. The Explorative Project "Peptide Libraries on a Chip" under the direction of Dr. Ralf Bischoff is undertaking a new approach to facilitate highly complex peptide arrays. The individual amino acids are first embedded in a solid carrier material (e.g. diphenyl formamide), which is subsequently processed into well-defined microparticles. These microparticles are then addressed to defined locations on the chip via electrostatic interactions. Not until all 20 amino acids have been distributed – and each field is addressed with an amino acid – are the amino acids firmly bound. For this the carrier material is melted and the amino acids are released, thus forming the peptide bonds. After several washing and drying steps, the next synthesis cycle can be performed and a further amino acid can be added to the growing peptide chain. A great advantage of this method is the possibility to wait until all 20 different amino acid particles are distributed to their designated location on the chip before the formation of the peptide bonds has to be carried out. Compared with various lithographic methods (see box), significantly fewer individual reactions are required. Thus the probability of faulty synthesis is reduced.

The highly specific addressing of amino acids to the corresponding spots on the chip is ensured via electrostatic forces: Initially, the amino acid particles (consisting of e.g. the amino acid threonine and the carrier material surrounding it) are electrically charged in such a way that all particles have the same charge. The spots on which the threonine particles are to be affixed are provided with the respective countercharge. The charged particles are then brought into contact with the chip via aerosol delivery and attach precisely at the selected spots (see figure). However, before the chip is brought into contact with the next amino acid particle containing aerosol (e.g. isoleucine particles), the charge pattern of the chip is varied: Now the sites on the chip are provided with a countercharge where the amino acid isoleucine is to bind (see figure). In total, this cycle is repeated 20 times – once for each of the 20 amino acids. The particles are subsequently melted, and the peptide bonds are formed for all 20 amino acids simultaneously. The team at the German Cancer Research Center (DKFZ) uses a semiconductor chip comprising an array of individually addressable pixel electrodes as support for the peptide arrays. By applying voltages to the electrode array, any arbitrary charge pattern can be generated and varied on the chip. "One of our goals is to improve this chip. We want to design highly complex chips that consist of up to 40,000 pixel electrodes per cm^2. The complete proteome of a bacterium could find room on such a chip," Ralf Bischoff explains.



Chip

PROJECT LEADER
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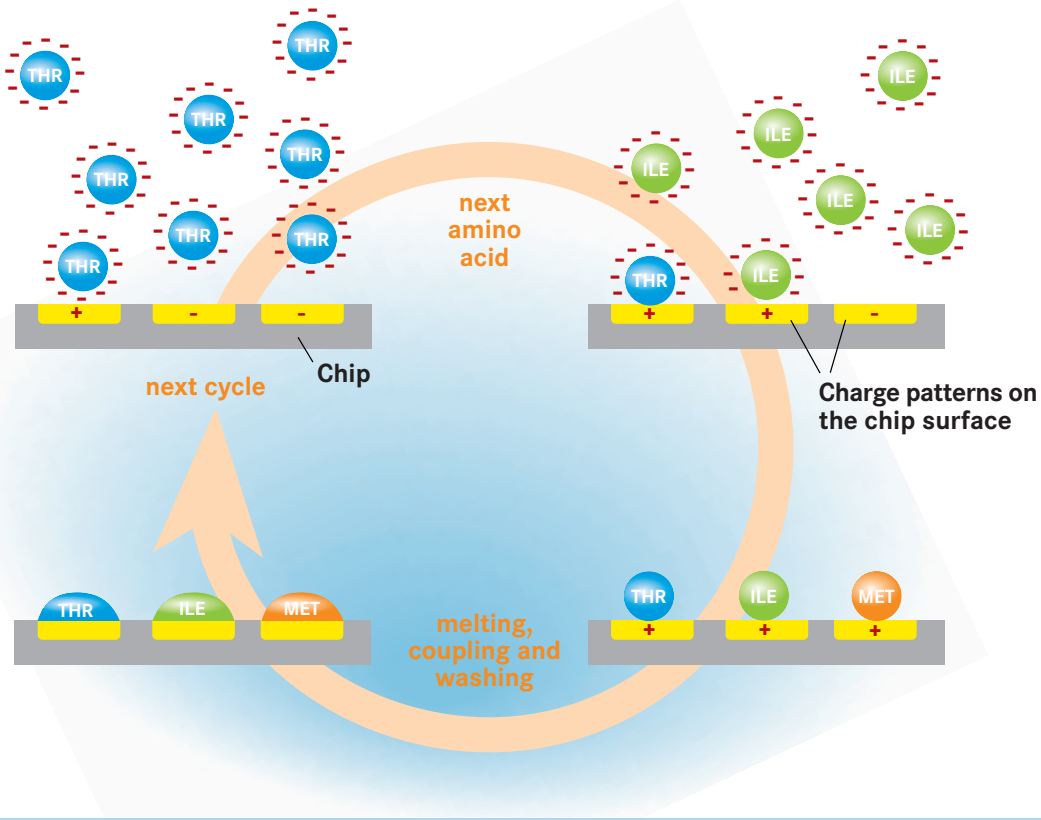

Deposition of amino acid particles on the chip using electrostatic forces. Charge patterns on the chip surface are generated and configured by the microchip itself.

PRODUCTION OF DNA ARRAYS

PHOTOLITHOGRAPHIC METHOD

The photolithographic method enables synthesizing oligonucleotides directly onto the surface of the chip. For this approach a support is used on which the individual spots are chemically protected by light-sensitive protective groups. When these protective groups are radiated with light, they are destroyed, and the spot is deprotected. The deprotection of the spot ensues through lithographic masks which only let the light pass at clearly defined locations. In the next step the first nucleotides are added (e.g. dATP) that in turn also have a protective group. They bind selectively to the spots whose protective groups have been destroyed. Before the second nucleotide (e.g. dCTP) comes into contact with the chip, an additional exposure – via a new mask – is implemented. Thus, exactly those spots are deprotected to which dCTP is now supposed to bind. This cycle is conducted for all four nucleotides, so that after the fourth cycle all spots have a nucleotide bound to them.

The nucleotides that are used for synthesis have the same light-sensitive protective groups as found on the support. This prevents the subsequent nucleotides from coupling to the wrong spots.

MODIFIED INK JET PRINTERS

Using modified ink jet printers, nucleotides can be transferred directly to the synthesis spots. Instead of the colors cyan, magenta, yellow and black, the nozzles disperse the nucleotides dATP, dTTP, dGTP and dCTP. Using this method, already synthesized DNA samples can also be transferred to the chip. The disadvantage with this application, however, is that all samples have to be synthesized separately beforehand.

MECHANICAL MICROSPOTTING

With this method a robot transfers the sample directly to the chip. Here, too, both nucleotides and finished DNA samples can be transferred.

Standardized procedures for high quality data

QUALITY MANAGEMENT IN THE NGFN

Successful collaboration depends on common standards. For scientists to share knowledge, they must be able to trust that all researchers are equally conscientious and conform to the same rules. This is especially true for a large network like the NGFN, which has around 350 projects from 80 scientific institutes and companies. For this reason, since the end of 2003 the NGFN has been building up a comprehensive quality management (QM) system. Quality management coordinators have been named, and currently seven working groups are concerned with QM in the following fields: genotyping; microarrays; production of open reading frame (ORF) resources; functional assays and cell cultures; proteomics and protein analysis; clinic management, data management and analysis; and animal models. Apart from assuring optimal data exchange, the QM measures aim to ensure that all materials and results produced within the NGFN maintain the same high quality. The respective quality standards follow international guidelines, if such exist.

STANDARDIZED TECHNIQUES AND QUALITY-CONTROLLED MATERIALS

Many work steps have already been standardized and documented for the entire NGFN network, including techniques to produce samples and to process microarrays. These standards improve the quality of the results and ensure the transparency of experimental procedures. They are intended to be used by as many partners as possible in the NGFN on the basis of voluntary self-commitment. Procedure descriptions are subjected to the respective QM working group for approval before they are made available on the NGFN intranet.

The NGFN also specifies strict quality criteria for raw materials such as DNA and RNA. "We ensure the good quality of DNA and RNA specimens by performing complete checks. For example, we test the concentration and the purity of the material with spectrophotometric analyses," explains Dr. Holger Sültmann, spokesman of the working group "Microarrays". "The quality of each individual microarray is carefully checked and documented following a standardized procedure."

In the production of ORF resources every clone is checked to make sure that it really contains the complete and correct ORF sequence. The National Genotyping Platform (NGP) performs a validation of the respective genotyping in the form of ring trials. The results of these ring trials are evaluated particularly with respect to reproducibility.

MANAGEMENT SYSTEMS FOR LAB INFORMATION

Several Systematic-Methodological-Platforms (SMP) have introduced lab data management systems. They ensure that all experiments are accurately documented. From the registration of sample entry to the scientific evaluation, all work processes must be documented without any gaps. For instance, within the SMP Cell, in the production of ORF resources, a management system assigns distinctive names for samples, thus creating a standardized nomenclature for the entire process sequence. In the SMP Protein a management system records different physical and chemical conditions for automated protein crystallization experiments. Within the NGP each microtiter plate has its own identifier code, corresponding to a linear bar code. This is linked via the plate coordinates to the sample IDs. Increasingly, moreover, sample test tubes labeled with a 2D barcode are used for incoming DNA samples which can then be pipetted onto the microtiter plates in an automated procedure.

DATABASE STRUCTURES AND INSTRUMENTS FOR DATA INTEGRATION

The QM working group "Clinic Management, Data Management and Analysis" has developed standards for describing clinical data. Christian Lawerenz of the German Cancer Research Center (DKFZ), quality coordinator of the group, says, "Now we are able to transfer data within the NGFN and build databases in which patient records involving different disease pictures are merged. The core parameter set includes attributes such as age, diagnosis, sample type and time taken that are relevant for all disease networks. These basic data are supplemented by specific parameter sets for the individual indication areas. Together with the users, we continually develop these parameter sets further." To ensure smooth data transfer, all participants must use a common vocabulary. "That is why NGFN ontology is rendered in a special XML-based language, the Web Ontology Language (OWL)," Christian Lawerenz explains. Due to the semantic description capabilities of OWL, the ontology is presented exactly on the basis of rules and terminologies and can serve as template for the data transfer. The NGFN ontology is also integrated into the NGFN portal for high-throughput technologies (MIPS Express). Important NGFN databases, such as the epidemiological metadatabases in Munich, Kiel and Bonn, the heart/cardiovascular database in Munich and iCHIP as local database platform for various Disease-oriented Genome Networks, have already been integrated into the NGFN ontology.

However, all these measures do not suffice – quality management in the NGFN is a continuing process. To further optimize the quality and reproducibility of the research results, additional quality assurance strategies are being developed in regularly scheduled workshops. Publishing the QM results is also being considered. "In this way we want to stimulate a broader discussion on the subject," explains Professor Stefan Schreiber, spokesman of the NGFN Project Committee. "Due to the diversity and the large number of leading experts in the field of disease-oriented genome research and in the platforms, the NGFN could thus make a contribution to elevating quality standards in German genome research overall."

Material check

DISCOVERING AND MARKETING KNOWLEDGE – TECHNOLOGY TRANSFER COORDINATION IN THE NGFN

"Know-how and inventions often slumber unused in the researchers' laboratory books," Dr. Isabel von Korff says. "Worldwide only five to seven percent of the patents from scientific research institutions are utilized commercially." Dr. von Korff, who is a natural scientist with several years of experience in the pharmaceutical industry, is director of the Technology Transfer Coordination Office in the NGFN (KTT). This office coordinates the activities of the more than twenty technology transfer offices that are involved in the NGFN and sees to that the results from NGFN-research are commercialized. "Intellectual assets have to be managed actively just like any other assets to make a profit," she explains.

Since the German Federal Ministry of Education and Research (BMBF) started its commercialization initiative in 2001, much has changed in the German technology transfer landscape. Alongside technology transfer centers (German abbreviation: TTE) that manage the intellectual property of research institutions (e.g. Ascenion for life science centers in the Helmholtz and the Leibniz Associations, or Garching Innovation GmbH for the Max Planck Society), a nation-wide network has since emerged comprising 22 patent and licensing agencies. These agencies (German abbreviation: PVA) are private companies commissioned by universities which manage the patenting and commercialization of the research results from all disciplines. The agencies are organized regionally and work on commission for all institutions of a region. "For that reason, a PVA team must simultaneously represent the interests of a mechanical motor, a substance used in making porcelain, an asthma drug, or a microchip," Dr. von Korff explains. In recent years a number of technology transfer agencies have been founded that specialize in a certain research area. One example is Ascenion GmbH, which evolved out of an initiative of several Helmholtz institutes and which concentrates on the life sciences.

FROM ASSESSMENT TO COMMERCIALIZATION

The new Technology Transfer Coordination Office in the NGFN links the many different groups involved. Already at the start of the second NGFN funding phase, all scientists were obligated to make a binding choice of the transfer facility they wanted to work with in the future. The project managers were free to choose whether to stay with the regional technology transfer center or to accept the offer of the Technology Transfer Coordination Office in the NGFN. "This way clear competences were created from the beginning," Isabel von Korff says. The legally binding collaboration always includes all of the steps from an initial assessment to commercialization. She continues, "We want to avoid that some offices only pick out the raisins in the cake and just shuffle off the economically less interesting projects after assesment."

THE "GENOME MARKETPLACE"

Since May 2005 the Internet platform www.genome-marketplace.de provides potential industry partners with access to interesting results from the NGFN which could be commercialized. "To accomplish this, we implement our principle of presenting one face to the customer," Dr. von Korff explains. On our website we offer patented inventions and technologies, but also laboratory and research materials. Interested companies can contact the relevant technology transfer office through the NGFN Coordination Office. The genome marketplace evolved out of the numerous coordination processes within the network. These ensure that all research results in the second funding phase of the NGFN are analyzed to meet defined high quality standards and are patent protected by suitable intellectual property rights. Among other services to facilitate this, the Technology Transfer Coordination Office provides a central publication screen. Then the research results are offered to industry via the genome marketplace website. In addition, continuing education symposia are organized for scientists in the field of Intellectual Property (IP) Asset Management. In individual cases, the NGFN Coordination Office provides further services, particularly for commercial exploitation of IP to technology transfer centers, if this is expressly requested. For example, this could be the case when special industry know-how or experience in founding and assistance with spin-offs is needed. "Our concept enables a close-meshed network of research and industry," Dr. von Korff adds. "That is the best precondition for an efficient and successful technology transfer."

In November 2005 Ascenion established a collaboration with BioDeutschland. The new biotechnology industry association already has more than 80 member companies. This will ensure a close and efficient link to the German biotechnology industry, thus leading to more efficient and faster commercialization of NGFN technologies in Germany.

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