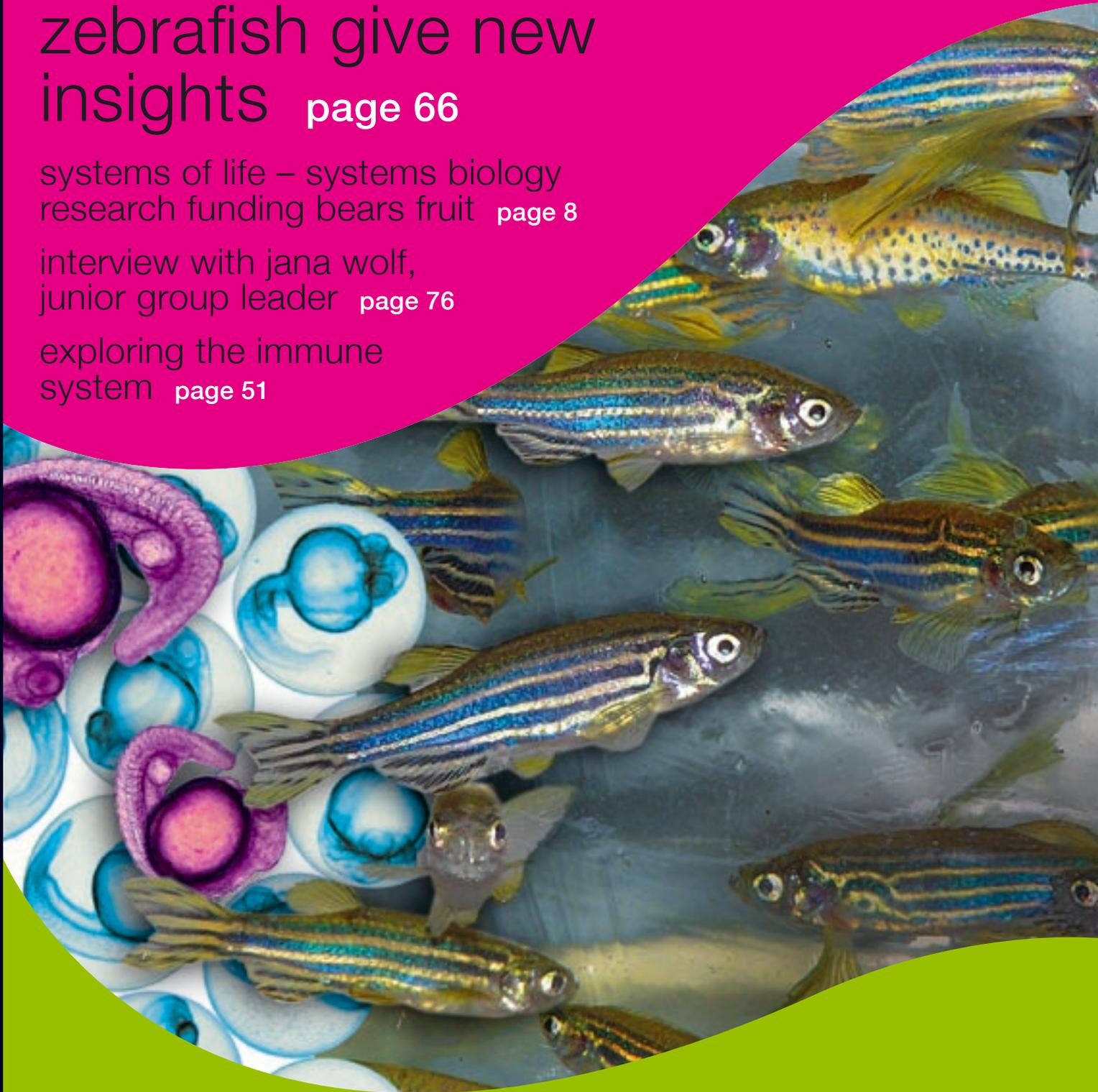


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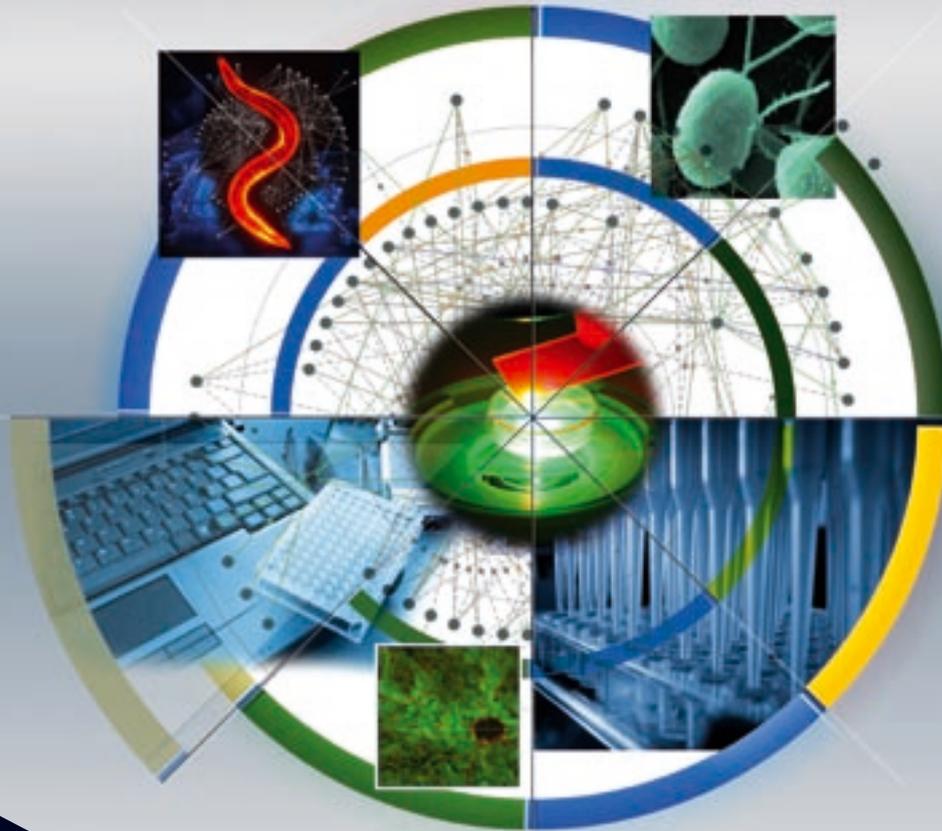
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Systems biology is a young and dynamic discipline that sees the whole picture. As part of the life sciences it builds a bridge between sophisticated laboratory experiments and mathematical modeling, between high-tech data measurements and computer-aided data evaluation. Its research subjects are the network-like entangled activities of signal transduction and metabolism in cells, tissues, organs and organisms. Systems biology research deals with this complexity by organising itself into interdisciplinary networks. Experience this fascinating, upcoming branch of science and what answers it provides to previously unresolved questions about human life in this international edition of the new magazine systembiologie.de.

Cover photo: Zebrafish and zebrafish embryos in different stages of development (Photo: Prof. Dr. W. Driever, Institute of Biology I, University of Freiburg)

welcome note

Dear Reader,



We are facing big challenges in the 21st century: Demographic developments, the rapid increase in common diseases, climate change, and finite fossil and energy resources require innovation for the future. With its High-Tech Strategy, the Federal Government aims to strengthen Germany's pioneering role in solving pressing global problems in the areas of climate/energy, health, mobility, security, and communication.

Systems biology is a key technology in the life sciences. It will play a crucial role as a driver of innovation in the areas of bio-economy and medicine over the next decade. Systems biology offers an opportunity to gain completely new insights into the dynamics and interaction of life processes. By combining molecular biology approaches with mathematical computer models, it enables us not only to gain an initial understanding of life processes but also to predict specific processes. This opens up completely new possibilities ranging from applications in agriculture to the development of customized drugs with reduced side effects.

The Federal Ministry of Education and Research (BMBF) recognized the innovation potential of systems biology and its importance for science and industry in Germany at an early stage. We have supported research in this field with funds of altogether more than €300 million over the past ten years. We will continue to increase Germany's importance as an international leader in systems biology by establishing innovative research structures, pooling national expertise and supporting young researchers.

As a young discipline, systems biology brings together researchers from the areas of biology, chemistry, medicine, physics, computer science and mathematics and from experimental and theoretical science. The magazine systembiologie.de is a new platform where they can exchange information. Researchers and companies will provide easy-to-understand information about their results and explain the use and potential of this highly innovative discipline. systembiologie.de addresses interested readers from science, industry, politics and the general public.

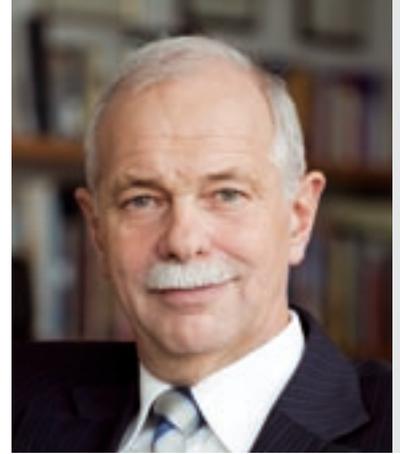
Enjoy your reading!

A handwritten signature in blue ink that reads "Annette Schavan".

Prof. Dr. Annette Schavan, MdB
Federal Minister of Education and Research

welcome note

Dear Reader,



The Helmholtz Association tries to find answers to the major issues that affect people today and will continue to affect them in the future, whether in relation to energy supply, the sustainable use of natural resources, mobility, or the treatment for incurable diseases.

In healthcare research especially, we face enormous challenges. Rising life expectancy and steadily falling birth rates are accompanied by an increase in chronic age-related illnesses. They include degenerative diseases of the nervous system and the skeleton, cancer, cardiovascular and metabolic diseases, pulmonary diseases and chronic inflammatory complaints. In addition, changes in the way we live that are characterised mainly by poor diet and lack of exercise have led, especially in Western countries, to a steep rise in metabolic disorders such as diabetes. Finally, increasing global mobility greatly facilitates the spread of infectious diseases.

Together with our university partners, Helmholtz Association scientists are helping to decipher the causes and genesis these frequently complex disorders and develop new strategies to prevent, diagnose and treat them. Our research rests on three pillars:

- **Excellent basic research**
- **Analysis of complex biological systems**
- **Translation of research findings into clinical application**

In the Helmholtz Association, systems biology is set up as a multidisciplinary field of research, in which primarily cellular processes are analysed at the molecular level so that predictive, mathematical computer models can be developed. In the future this will enable a better understanding of how diseases originate and customised treatment can be made possible. To turn this new scientific approach into an internationally visible “lighthouse” of German research, six Helmholtz Centres have joined together with university and non-university partners to form the Helmholtz Alliance on Systems Biology. The Helmholtz Association is providing €24 million in additional funding for this research up to 2012.

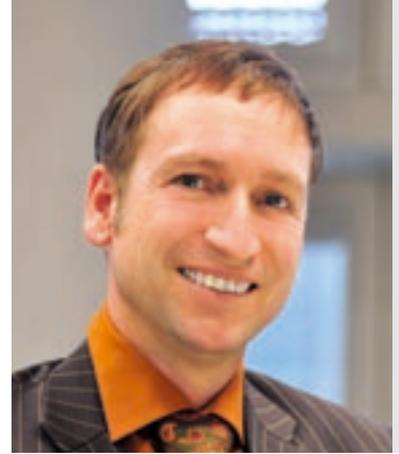
In the *systembiologie.de* magazine, scientists from a wide range of disciplines have united on a platform that is of interest not only for the science community, but for politics and society as well. This is very much in keeping with our mission, namely to help solve the major and pressing issues facing society, science and industry.

A handwritten signature in blue ink, which appears to read 'Jürgen Mlynek'. The signature is stylized and fluid.

Yours, Prof. Jürgen Mlynek

President of the Helmholtz Association

we are the champions of the world!



Editorial

These were the words Jürgen Klinsmann used six years ago as he set about making a lasting change in the bleak view that Germans held of themselves with regard to football. Two years later, as we now know, this led to a fairy-tale summer in Germany, where – and who would have thought it possible in the country of poets and thinkers but also a nation that loves the songs of rock star Herbert Grönemeyer – we may not have won the World Cup but very much felt that we were the champions of the world.

In systems biology we face a similar phenomenon. Accustomed to constantly trailing the United States in the life sciences, we might easily be content to emulate the US and be the eternal second-best in systems biology too. But a glance behind the scenes in systems biology soon reveals that Germany is an international front runner in this young field of research. In the early years of this millennium a discreet group of scientists met over a number of weekends at an idyllic spa hotel near Frankfurt to devise ideas for a national systems biology support programme in a creative but complex process. That led to the invitation to tender for HepatoSys, Germany's first systems biology initiative, and that was at a time when Europe was still in a deep systems biology sleep and only the first large programmes were being launched in the United States.

Today, less than ten years later, we in Germany can look with justified pride on a thriving systems biology landscape in this country. The German Federal Ministry of Education and Research alone is currently funding a series of systems biology initiatives to the tune of around €30 million a year. In concerted action by the federal and state governments four national systems biology lighthouses have been created. These activities are complemented by the Helmholtz Alliance on Systems Biology, which interlocks systems biology research at the Helmholtz Association's healthcare centres and university research in a remarkable way. In several international surveys conducted in recent months the Helmholtz Alliance and other initiatives were classified as being involved in cutting-edge international research. Germany's young, dynamic systems biology culture is regarded in other countries as internationally exemplary.

We have used this opportunity to publish the magazine *systembiologie.de*. Our aim is to present readers with exciting stories, news and notable key players in German and international systems biology. The magazine will initially be published biannually in German language. About once a year the International Edition, the first edition you are holding in your hands, will present highlights of systems biology research from Germany and beyond.

In 2011, Germany has the honour to host the 12th International Conference on Systems Biology ICSB in Heidelberg and Mannheim. We cordially invite you to attend this premier conference in systems biology and to experience Germany's world-known hospitality.

We wish all our readers an entertaining reading and an exciting insight into the fascinating world of systems biology.



Yours truly, Roland Eils, for the editorial team

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systems of life – systems biology

Research funding bears fruit

By the editorial team

Systems biology: an (un)beloved child of biology?

“The feelings that a majority of people have about mathematics are those that Aristotle felt tragedy should awaken, namely pity and fear. Pity with those who have to toil over mathematics and fear that one might one day also find oneself in this dangerous predicament.” The feelings that the broad mass of the population have about mathematics could not be expressed better today than they were by Paul Epstein, a mathematician renowned for his contribution toward number theory, at the beginning of the twentieth century. The bioscientists as representatives of a discipline that has so far been largely free of mathematics were initially no exception. So it is easy to understand why systems biology as an upcoming discipline dedicated to the systemic recording of complex biological circumstances was not welcomed unreservedly within the biosciences. Indeed, early reactions noted that systems biology was merely old wine in new bottles. Biology had, after all, always seen itself as a systemic science. Systems biology, however, is accompanied by a radical paradigm shift in bioscience research. It sees itself as a strictly quantitative science that is always linked with setting up mathematical theories.

What does systems biology stand for?

The term systems biology consists of the words systems and biology. Systems theory, to which the first word refers, has its origins in Karl Ludwig von Bertalanffy, 1901–1972, who published an essay on systems theory in 1949 (Bertalanffy, 1949). According to von Bertalanffy, the principal objective of systems theory is to find generally valid laws in systems and to formulate them with scientific accuracy. Today the concept of systems theory, which has since been constantly expanded, is used as a matter of course in both science and the humanities. This widespread use also explains why it is at times difficult to formulate a universal definition of systems biology. While some scientists define systems biology as an area of science that takes a comprehensive look at the interaction between components of biological systems and their interaction in the context of an organism, others even see it as a paradigm shift within the entire scope of the life sciences. According to this view, systems biology, in contrast to the previously customary approach in molecular and cell biology, is less a matter of research into details and strictly delimited issues than an attempt to merge existing knowledge, new facts and data

into an overall mathematical model in the context of the overall organism under investigation (Noble, 2006). Still other scientists see systems biology as a discipline that uses high-throughput methods to clarify systematically the networking of individual biological building blocks and thereby arrive at a holistic, in-depth understanding of biological processes. In contrast, a definition that is frequently used sees systems biology as a discipline that simply applies the methodology of mathematical systems theory to issues of modern molecular biology.

A systemic methodology is not entirely new in the life sciences. In physiology, pharmacology, biochemistry and other biology disciplines, concepts of mathematical modelling have long been in use. A well-known case in point is the work of Reinhart Heinrich, considered one of the founders of metabolic control theory in biochemistry (Höfer, 2007).

Indisputably, the current boom in systems biology is mainly a consequence of the triumphant progress of high-throughput technologies in biology. It took the abundance of molecular biology data generated with the aid of these technologies to lay the foundations for investigating these details in their dynamic interplay by means of mathematical models.

Systems biology programmes and initiatives in Germany: A success story that will soon celebrate its tenth birthday

It is a striking fact that systems biology, in spite of all reservations, has become an incomparable success story in the few years since it was launched in Germany. Once it had kicked off, the process of theorising biology seems to be irreversible and is making swift progress.

To position Germany at the forefront of international research, the German Federal Ministry of Education and Research (BMBF) published the “Systems of Life – Systems Biology” funding concept in 2001 as a result of a process of discussions with high-calibre experts. With this publication the BMBF sought to “establish systems biology in Germany and open up its potential for future research and development in science and industry”. Choice of and support for research projects are made and provided by an

international steering committee and led to the setting up of an interdisciplinary German competence network focussing on research work on the liver cell model system. The competence network HepatoSys – Systems Biology of Hepatocytes was funded from 2004 to 2010 and has developed in a short time into an internationally recognised research network. HepatoSys thereby long played a leading role in systems biology research in Germany. The first three-year funding phase with a financial volume of € 14 million and 27 workgroups was followed in 2007 by a second funding phase with a volume of € 22 million. At this stage over 40 partners were already involved. The second funding phase ended in spring 2010 and since then it has been continued by the Virtual Liver initiative (cf. article on p. 64).

German systems biology research enters the international stage: The 2004 International Conference on Systems Biology (ICSB) in Heidelberg

Since 2000, the International Conference on Systems Biology (ICSB) is held annually by the International Society for Systems Biology (ISSB). In 2004, it was first organized by the German systems biology community in Heidelberg. The ICSB is a worldwide forum for scientific debates and makes a major contribution towards ensuring that systems biologists from all countries meet and form alliances. With over 800 participants the 2004 ICSB in Heidelberg got off to an extremely successful international start (Schuster, Eils, & Prank, 2006). Further, the HepatoSys competence network initiated by the BMBF organizes the Systems



In 2011, the Conference Center Rosengarten in Mannheim will be the main ICSB venue (Photo: m:con)

Biology of the Mammalian Cell (SBMC) conference as a biannual international event of its own that is held at different locations in Germany. In 2011, Germany has once more the honour to host the 12th ICSB in Heidelberg/Mannheim and we cordially invite you to attend this most important conference in systems biology (see Events on p. 81).

German systems biology research outgrows its children's shoes: The Helmholtz Alliance on Systems Biology and FORSYS – Research Units for Systems Biology

Almost simultaneously in 2006, the Helmholtz Association as the largest German research organisation and the BMBF launched two more systems biology initiatives and German systems biology research finally outgrew its children's shoes. The Helmholtz Alliance on Systems Biology is creating a strong and resilient systems biology research landscape within the Helmholtz Centres jointly with university research facilities. The network is funded with around € 50 million provided in equal measure by the Helmholtz Association's Initiative and Networking Fund and the Helmholtz Centres involved. The Alliance's main focus is on research into the background to complex human diseases and the cellular compounds, organs and organ systems affected by them. An interesting aspect is that the Helmholtz Centres involved had already previously played a leading role in many national genome research initiatives. On the basis of this expertise the Helmholtz Alliance on Systems Biology has been able to integrate its expertise in genome research, molecular cell biology and bioinformatics and to extend it by setting up new workgroups for mathematical modelling.

The BMBF initiative "FORYS – Research Units for Systems Biology" aims to set up under one roof systems biology research units organised along interdisciplinary lines and makes a fundamental contribution toward strengthening the current systems biology research infrastructure in Germany. At the same time FORSYS seeks to support junior research scientists (by setting up a total of nine junior research groups) and by creating training opportunities for systems biologists. As part of the FORSYS initiative four centres were set up in Freiburg (FRISYS), Heidelberg (ViroQuant), Magdeburg (MaCS) and Potsdam (GoFORYS) with € 45 million

in combined funding over a five-year period. The outstanding characteristic of FORSYS is the variety of scientific issues that it deals with.

The downstream FORSYS partner programme complements the FORSYS initiative especially by incorporating university, non-university and private enterprise research capacities that are outside the scope of the present four FORSYS centres. FORSYS partner programmes also assist other junior scientists in setting up independent junior research groups. At present, the BMBF supports ten cooperation projects and twelve junior research groups as part of the FORSYS partner programme. Another systems biology centre funded by the BMBF and the Berlin Senate is the Berlin Institute for Medical Systems Biology (BIMSB), an extension of the Max Delbrück Centre for Molecular Medicine (MDC) in Berlin-Buch that is operated jointly with Charité – Universitätsmedizin Berlin. The next issue will include a detailed profile about BIMSB.

Implementing research findings for the benefit of the patient: systems biology in medicine

With a view to leading systems biology out of basic research and into a more strongly application-oriented context, the BMBF launched two more systems biology funding programmes in 2008 namely MedSys – Medical Systems Biology and GerontoSys – Systems Biology of Aging. A crucial consideration in setting up the MedSys and GerontoSys initiatives was that new insights into complex diseases such as neurodegenerative disorders or cancer cannot be gained efficiently enough by means of conventional genome-based research alone. An integrated systems biology approach that records, analyses and evaluates the individual factors and multifactorial aspects that lead to the specifics of a complex disease holds the promise of success.

Consequently, the BMBF defined the target of GerontoSys as research into mechanisms that cause the aging process and called for the findings to be placed in the context of an overall picture. The relevant biological factors that contribute toward

Systems biology initiatives in Germany

Project	Funding period	Funding volume (€ million)	Research networks funded	Website
HepatoSys I ¹	2004-2006	14	5	www.hepatosys.de
HepatoSys II ¹	2007-2010	22	6	www.hepatosys.de
Virtual Liver Network	2010-2015	43	1	www.virtual-liver.de
QuantPro ¹	2007-2011	23	14	www.fz-juelich.de/ptj/quantpro
SysMO ¹ / SysMO2	2007-2013	20	11/7	www.sysmo.net
FORSYS ¹	2007-2011	45	4	www.forsys.net
FORSYS-Partner ¹	2007-2012	32	10 (+12 junior research groups)	www.forsys.net
MedSys ¹	2009-2012	40	18	www.bmbf.de/foerderungen/12066.php
SysTec – New Methods of Systems Biology ¹	2009-2012	22	10	www.bmbf.de/foerderungen/12463.php
ERASysBioPlus ¹	2010-2012	8	16	www.erasysbio.net
Systems Biology of Aging (GerontoSys I) ¹	2009-2014	10	3 (1 competence centre)	www.bmbf.de/foerderungen/12744.php www.bmbf.de/foerderungen/14287.php
Helmholtz Alliance on Systems Biology	2007-2011	49	5	www.helmholtz.de/systemsbiology

¹ Source: BMBF: Impulsgeber Lebenswissenschaften - Forschung für die Innovation der Zukunft, 2009

age-related illnesses should then be identified and used to develop new diagnostic and therapeutic applications. Pharmaceutical and food industry companies are integrated into many projects as partners. Great expectations are placed on systems biology methods and research, especially in the area of developing medicinal active compounds. This is shown not least by the fact that many pharmaceutical companies already have departments and laboratories for this purpose (Butcher, 2005; Hopkins, 2008).

Systems biologists – Scientists with excellent future prospects

Systems biology research combines molecular and cell biology with mathematics, physics and the engineering sciences. This places particular demands on the scientists involved. Up until now, most research scientists working in systems biology have received conventional education in one discipline or another and then worked their way into complementary research fields. The high level of interdisciplinarity and the complexity of this learning process explain why skilled, well-trained systems biologists with knowledge of biological, mathematical and programming disciplines are in great demand (Aderem, 2005). It is also why it is widely felt that the shortage of qualified scientists is a bottleneck in systems biology research (Bialek & Botstein, 2004; Wingreen & Botstein, 2006). Against this background it is clear why all of the initiatives mentioned provide effective assistance and training for junior scientists. As part of the FORSYS programme, for example, new curricula for bachelor's and master's courses in systems biology have been established and newly conceived doctoral programmes have been created (cf., for example, the Potsdam GoFORSYS training programme on p. 19 of this issue). These programmes are complemented by summer schools and other events that cover active scientists' in-service training requirements.

A paradigm shift in biology?

If systems biology is seen as a process of theorising the biosciences and thus as a paradigm shift in biology, the future of systems biology can only be its own disintegration as a separate discipline. Our postulate is that in the future it will be impossible to imagine biology without theory formation and that no biological experiments will be carried out anymore without first consulting a computer for the optimal experiment design. It remains to be

seen whether this degree of theorisation is achieved in the biosciences within ten years or whether it will take much longer.

Numerous obstacles will certainly need to be overcome en route. The BMBF is already providing lasting support for the process in the shape of further funding (cf. table, p. 10) and in planning future measures.

In the future, biology will indisputably differ totally from the way it is taught at our universities across the country today. After all, as Galileo Galilei wrote well over 400 years ago, "The book of nature is written in the language of mathematics." Biology's days as the only scientific discipline that is largely free of mathematics are numbered.

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mathematics against viruses

The ViroQuant Modelling Research Group

By Lars Kaderali

With HIV, hepatitis, influenza, etc., viruses are on the advance all over the world. And due in no small part to bird and swine flu, viral infections are also a topical subject for public debate once more. At Heidelberg University's BioQuant Center a young interdisciplinary team is engaged in research to gain a better understanding of viral infections. The researchers combine mathematical models with machine learning and data mining and apply these methods to experimental data. In this way they hope to learn more about how viruses multiply and about the cell's immune response. In addition, they are engaged in a systematic quest for points of attack for new drugs.

Viruses consist of just a small number of proteins and their viral genome, a DNA or RNA molecule that contains the construction manual for building new virus particles. Without its host cell the virus can neither multiply nor spread successfully. In a viral infection the virus first smuggles its genetic construction manual into the host cell and uses the host's cellular mechanisms to produce new virus particles.

To do this, the virus misuses cellular resources and processes and thus multiplies at its host's expense. These processes are based on extremely complex interaction between the virus and the host cell that we are only just starting to understand for most viruses.

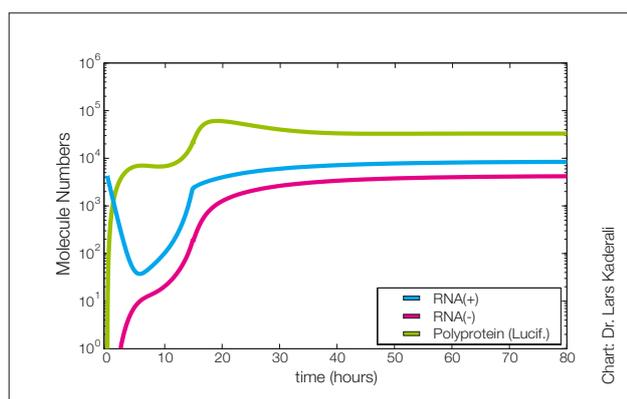
In contrast to the way in which bacterial infections are treated with antibiotics that "simply" launch a systematic attack on bacterial structures and processes, such a strategy is much less effective against viruses. By virtue of the typically very swift replication and high mutation rate of viruses, they are quick to develop strategies by which they can circumvent antiviral intervention. Therefore, an alternative strategy for antiviral drugs is to interfere in the interactions between the virus and its host cell, essentially by targeting a host process required by the virus.

However, this has to be done at points maximally affecting the virus, without causing lasting damage to the molecular processes in the host cells and thereby triggering serious side-effects in the host organism. A comprehensive understanding of the entire viral infection cycle and viral interactions with the host cell is thus required in order to develop new and innovative prevention and treatment approaches to treat life-threatening viral infections such as Aids.

Race between virus and immune defences

At Heidelberg University's BioQuant Center, new approaches are being taken in order to fully understand viral disorders using systems biology and to find new target molecules for antiviral drugs. Viruses are perfect examples of the need for research approaches based on systems biology because they can only be understood in the context of the host cell with which they interact in a varied and complex way. "We develop mathematical models that give us an exact description of virus replication in

Computer simulation of the intracellular replication of the hepatitis C virus genome in liver cells:



The simulation shows how the viral plus RNA strand (the blue curve) transfected at time 0 in HuH-7 cells is initially broken down quickly. At the same time, the RNA is transformed into protein (green) which in turn induces the synthesis of minus-strand viral RNA (red) which then serves as a template for the synthesis of new plus-strand genomes. After about eight hours an explosive increase in the concentration of viral RNA is observed in the cell, reaching a stationary condition in about 30 hours.



Dr. Lars Kaderali heads the ViroQuant Modelling Research Group at Heidelberg University's BioQuant Center since 2007.

the cell and the cell's immune response to the infection," explains Dr. Lars Kaderali, who heads a junior research group on the systems biology of virus-host interaction at the BioQuant Center. The researchers model both the virus multiplication and the cell's immune response on computers and are thereby able to replicate the race between the virus and the host's immune system.

Before the research scientists led by Lars Kaderali can develop mathematical models, however, they must identify essential host factors involved in the virus's lifecycle. They do this by conducting what are known as RNA interference experiments that enable them to target individual genes in live cells and silence them. Virus-infected cells are tested to find out which genes and thus which cellular processes the virus needs in the host cell in order to multiply. When the host factors required for propagation are removed from virus-infected cells by means of RNA interference, this can be seen in reduced virus replication.

In a large-scale interdisciplinary RNAi screening approach, researchers test thousands of genes as part of the BMBF-funded ViroQuant FORSYS project and measure how well different viruses are still able to multiply after the host gene in question has been silenced. In cooperation with virological partners, experiments of this kind are conducted on different viruses in Heidelberg. Along with HIV (the virus that causes Aids), the hepatitis C and dengue viruses are being examined. In further, even more targeted screening, individual processes in the cell are also being investigated more closely, such as in the congenital or acquired immune system, or intracellular transport processes that different viruses make use of.

Which host genes does the virus need?

The amount of data gained in genome-wide high-throughput experiments is so large that manual evaluation is no longer possible. Lars Kaderali's research group has therefore developed an automated pipeline to process the data statistically and identify

what are known as hit genes, i.e. genes whose silencing has a significant inhibiting or retarding influence on virus replication. Using bioinformatics methods, these hit genes are then mapped to cellular processes in order to identify important mechanisms that the virus makes use of in its lifecycle. This involves searching for both processes that all of the viruses investigated have in common and virus-specific processes. In these experiments the researchers have already succeeded in identifying several hundred interesting candidates that are currently being subjected to further investigation in functional experiments by virological workgroups.

"Close cooperation between biological, clinical and mathematical workgroups is essential. Without it a project of this kind could not be implemented successfully," says Kaderali, who holds a PhD in computer science from the University of Cologne. After research in the United States and post-doctoral research at the German Cancer Research Center in Heidelberg, Kaderali has been head of one of two junior research groups in the FORSYS project ViroQuant since 2007. His group is suitably interdisciplinary, with mathematicians, bioinformatics specialists, chemical engineers and physicists working together with biologists, virologists and medical specialists in data analysis and mathematical modelling.

Identifying candidate genes is only one of the first steps in a comprehensive systems-based virological approach, however. Only in small number of cases does merely knowing the identity of some of the host genes involved provide a direct explanation for underlying cellular processes that are often still completely unknown or characterised only in a very rudimentary way.

Computing power for the 'virus model'

The researchers face the problem of arranging the genes they have identified into molecular networks. This is a gigantic puzzle in which countless building blocks must be pieced together to make up an image. Here too, computer-aided calculation is employed. Using algorithms from machine learning, the virus puzzle is put together piece by piece. In principle the procedure is very similar to the one used in engineering to interrupt individual components in an unknown circuit and infer from the effects observed how the circuit functions overall. The mathematical processes employed involve intensive computing and are a fine example of the convergence of biology, medicine, mathematics and computing. As a computer scientist Dr. Kaderali enjoys developing new algorithms to explain complex biological systems, and once in a while then blocks an entire cluster of computers for several days to solve a new molecular network problem. The researchers recently proved that this machine learning approach is successful. In close cooperation with the workgroup headed by the virologist Professor Ralf Bartenschlager they succeeded in reconstructing on a purely data-driven basis molecular processes of the congenital immune system in hepatitis C infections.

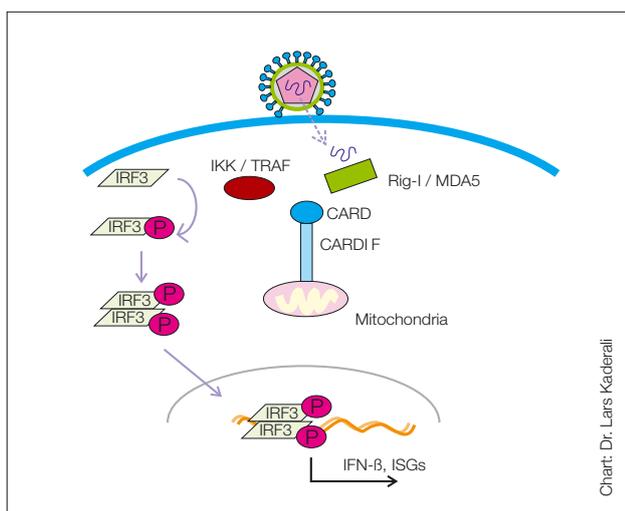
New points of attack for drugs

These machine-learning based processes are especially useful for a rough characterisation of new and previously unknown pro-

cesses between the virus and host cell. Once the basic structure is known and, in particular, once it is clear which reactions take place and which molecules interact with each other, a more detailed mathematical model of these interactions can be set up. What matters at this stage is the creation of a precise model, i.e. one that is quantitative and has a high resolution in time. Only then are dynamic computer simulations possible. "This mathematically precise description," explains Dr. Kaderali, "enables us to perform a computer sensitivity analysis and try out different scenarios. We can thereby find out which processes are critical in the race between the virus and the immune system. This in turn can provide valuable indications in terms of the points at which we can best attack using antiviral drugs."

In close collaboration with the Department of Molecular Virology at the Heidelberg University's Medical Faculty the researchers of the junior research group recently drew up a model of coupled differential equations that simulates replication of the hepatitis C virus in the cell in space and time. Using this model the researchers were able to reproduce the intracellular replication of the hepatitis C virus genome in the computer, achieving a high degree of conformity to data measured experimentally. At present the model mainly covers viral components, but it will gradually be extended to include host processes and the cell's immune response. By integrating processes of this kind into the replica-

Diagram of the Rig-I signal transduction pathway:



The Rig-I signalling pathway is a central component in the congenital immune response. Rig-I recognises alien (e.g. viral) RNA in the cell and leads eventually, via the activation of different kinases, to the phosphorylation of IRF3, which forms a duplex and moves into the cell nucleus. There, IRF3 activates the transcription of antiviral genes that both disturb virus replication in the cell and warn neighbouring cells of the infection. Viruses such as hepatitis C interfere with these immune processes by interrupting the signalling pathway and thus try to evade the cell's immune response. Quantitative and dynamic mathematical models of this process can reveal precise information about the kinetics and thus about the race between the virus and the immune system.



tion model using machine-learning procedures and data mining methods, a model of the entire hepatitis C virus lifecycle should take shape. Dr. Kaderali is hopeful that this will enable them to track down new target structures for drugs more effectively and quickly than in the past – by intervening at precisely the most sensitive points in the infection process.

The research project in brief:

Project name: ViroQuant – Systems Biology of Virus-Host Cell Interaction

ViroQuant is a systems biology research centre at Heidelberg University funded as part of the German Federal Ministry of Education and Research's FORSYS initiative.

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programmed cell death

Mathematical model provides new insights

By Inna N. Lavrik

Apoptosis, or programmed cell death, is crucial for multi-cellular organisms. Named after the Greek word for falling leaves, apoptosis does not cause any damage to the organism – in contrast to other types of cell death as, for example, necrosis. Every day about ten grams of cells perish in our bodies due to programmed cell death. Apoptosis is the prerequisite that enables an organism to develop, to differentiate specialised tissues and to eliminate harmful cells. For example, in the course of development of human embryo the cells between the fingers and toes die to generate normal fingers and toes. Also, as the nervous system develops, nearly half of the neurons die by means of apoptosis.

Programmed cell death is the result of a complex signal transmission. If the signal is faulty and “too much” or “too little” apoptosis takes place, illnesses occur. Degenerative diseases such as Alzheimer’s, Parkinson’s, Huntington’s and many autoimmune disorders, as well as Aids, strokes, ischemia and spinal cord damage are related to an excess level of apoptosis. Patients suffering from cancer or certain chronic complaints have, in contrast, a reduced apoptosis level. In cancer, faults in the apoptosis signalling pathway prevent the elimination of malignant cells. Uncontrolled cell multiplication is the result. The apoptosis process follows a complex yet highly ordered programme that sends the cell to its death. The cell shrinks, blister-shaped bulges form on the cell membrane, and the DNA breaks into pieces.

Caspases, the executors of the programme of death

Enzymes that cleave other proteins (known as caspases) play a key role in this process. All caspases are first created as catalytically inactive proteins. Without apoptotic signals they are present in the cell like many other proteins. If, however, the cell receives an apoptotic signal, the caspases are catalytically activated and are then capable of splitting more than 1,000 different proteins in the cell, leading very quickly to the cell’s self-destruction. Caspases are therefore the most important executors of the programme of death. This unique twofold function of the caspases and their ability to be activated by an apoptotic signal enable apoptosis to be set in motion by certain triggering factors.

How caspases are activated

What, then, are these apoptotic signals? Apoptosis can take place in two ways: by either an extrinsic (by means of a death receptor) or an intrinsic (mitochondrial) signalling pathway. The extrinsic pathway occurs when death ligands, members of the TNF (tumour necrosis factor) family, bind to their corresponding death receptors that belong to the death receptor family. This triggers the formation of a death-inducing signalling complex (DISC) in which so-called initiator caspases, are activated that then set the death programme in motion. The intrinsic signalling pathway can be triggered by a variety of factors, such as UV radiation, DNA damage or a lack of growth factors. All of these different stimuli lead to a mitochondrial depolarisation and the release of cytochrome c from the mitochondria. The release of cytochrome c from the mitochondria leads to the formation of a complex that is known as apoptosome and, like the DISC complex, results in the activation of the initiator caspases. Once the initiator caspases have been activated in the DISC or the apoptosome, they in turn activate effector caspases. Activation of the effector caspases leads to the above-mentioned fragmentation of the cell skeleton as well as the splitting of the DNA repair enzymes and many other proteins that are of essential importance for the normal cell programme. All of these changes have dramatic consequences and ultimately cause the cell to die.

Protection from accidental death

The cell does, however, have several ways of blocking apoptosis in order to prevent “incorrect” signals from triggering cell death. There are many apoptosis inhibitors, which can block the initiator or effector caspases. FLIP proteins inhibit initiator caspases in the DISC and XIAP proteins inhibit effector and initiator caspases at a later stage in the process. Furthermore, anti-apoptotic members of the Bcl (B cell lymphoma) 2 family can block the release of cytochrome c from the mitochondria and thereby prevent the formation of apoptosome and the onset of apoptosis. All of these blockers impede spontaneous cell death that can be caused by spontaneous activation of one caspase or another. Only a strong apoptotic stimulus that is capable of overcoming the effect of all these inhibitors is able to kill the cell.



Dr. Inna N. Lavrik investigates the signalling pathways of programmed cell death.

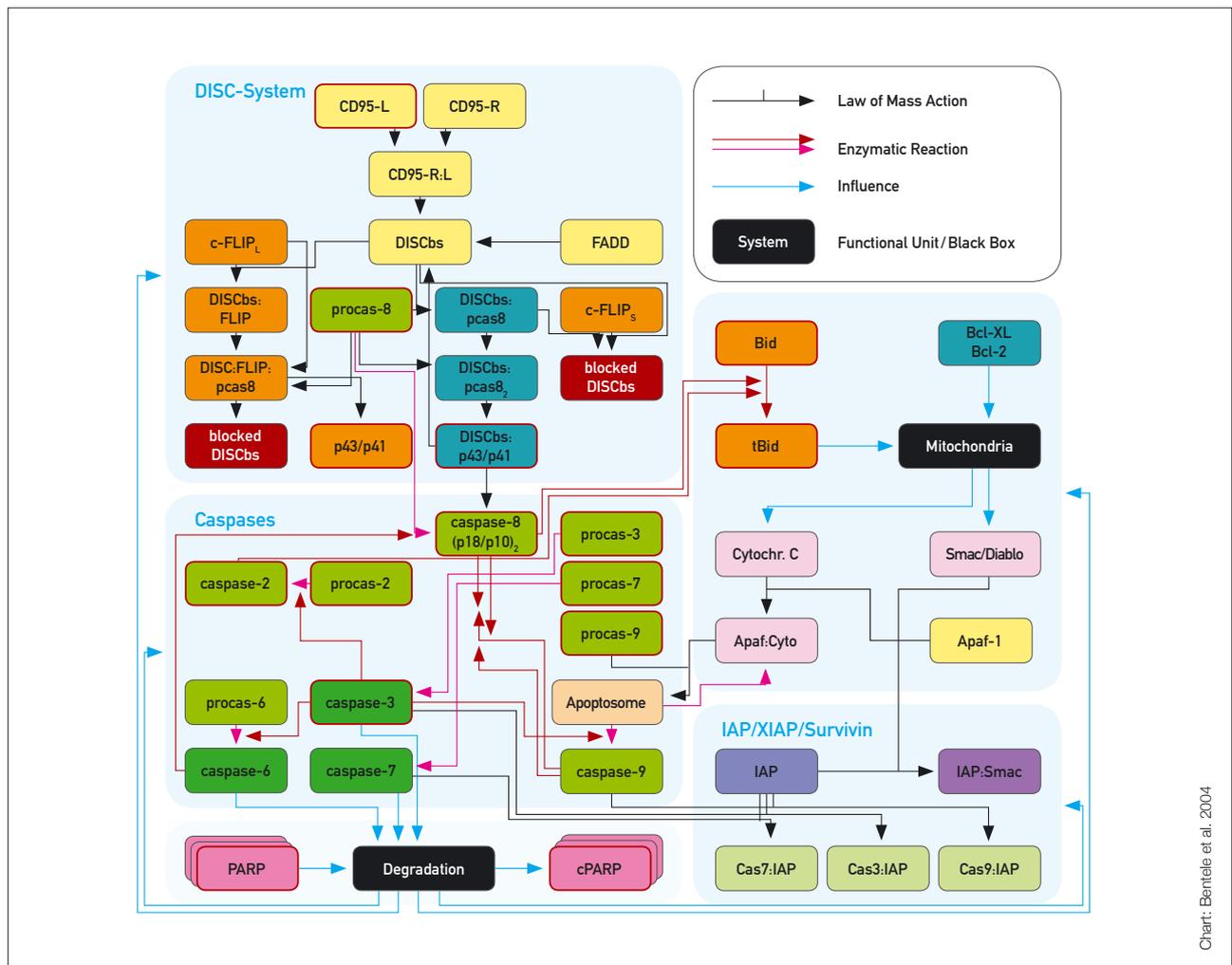
Levels, thresholds, signal strengths: where the systemic approach helps

This fascinating property of cells to kill themselves in accordance with a cellular programme that is clearly defined and regulated at many levels inspired the scientists to investigate apoptotic signalling pathways using systems biology methods. A cell can make a decision about life and death at several levels: at the level of death receptors, caspase activation or apoptosome formation. From the systems biology point of view it is interesting to know

whether there is a point of no return and which apoptotic signal strength leads to the death of the cell. To understand apoptosis systemically, we first modelled one of the prototypical death receptor signalling pathways, the CD95/Fas signalling pathway (Fig. 1).

The mathematical model was generated using ordinary differential equations (ODEs) and the biochemical reaction network was transferred to an ODE system. Each molecule of Ni in the ODE network was assigned a specific degradation or production rate $[dNi]/dt$.

Fig. 1: Mathematical model of receptor-induced programmed cell death (according to Bentele et al. 2004)



Our first mathematical model of CD95-induced apoptosis contained 41 molecules or complexes, 32 reactions and two black boxes. Reactions with well-known biochemical mechanisms such as those of the DISC system or the caspases were modelled mechanistically. For all other forms of interaction black boxes were introduced that are defined by their experimentally observed input-output behaviour. For black boxes such as mitochondria no prior knowledge of the exact underlying mechanism was assumed. The model was validated using a defined experimental set-up.

What is the point of no return? The model provides a clear answer...

The validated model provided a clear predicting capability, enabling to describe the real behaviour of the cell and the degree of caspase activation at different levels of CD95 stimulation. As the model also demonstrated, the CD95 signalling pathway shows a distinct threshold behaviour. This threshold is defined by the quantity of the FLIP inhibitor, which blocks activation of the initiator caspase in the DISC. A significant finding made by the model is that the decision about life and death is made at the CD95 DISC and the apoptosis can no longer be stopped once the initiator caspase has been activated in the DISC.

This initial systems biology model of CD95-induced apoptosis has proved to be a highly promising approach towards a better understanding of the signalling pathways and mechanisms of programmed cell death. Current systems biology research is aimed at developing strategies to sensitise cells to apoptosis. That could point the way to new cancer therapies. What are now needed are further experimental analyses and a link between them and the modelling. This would be worthwhile because it would open the way to controlling decisions made about life and death in both healthy and malignant cells.

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The research project in brief:

Project name: SBCancer: Systems Biology of Signalling in Cancer
The Systems Biology of Signalling in Cancer (SBCancer) research network is part of the Helmholtz Alliance on Systems Biology, an initiative to promote systems biology in Germany financed by the Helmholtz Association's Initiative and Networking Fund with which Helmholtz Centres, universities and other partners are associated.

www.helmholtz.de/systembiologie

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stepping stones to a career in systems biology

The GoFORSYS research network in Potsdam

By Susanne Hollmann and Carsten Müssig

As a science centre, Potsdam is extraordinarily attractive for students who are interested in life sciences. The courses of study at Potsdam University profit from being part of an in-depth network of high ranking, extra-university research institutions in which teams of scientists devote themselves to the burning themes of plant systems biology and molecular life sciences.

GoFORSYS, an alliance between Potsdam University, the Max Planck Institute for Molecular Plant Physiology and the Max Planck Institute for Colloid and Interface Research, is a successful example of this networking. GoFORSYS is one of four systems biology centres in Germany. Here, scientists are examining the connection between photosynthesis and biomass production. The regulatory networks of plant metabolism, which helps plant growth adapt to changing environmental conditions, is an additional focus of their research. The results are processed using methods from bioinformatics to finally map the cellular and physiological processes in computer models. A key aspect of the approach is the expansion of existing modelling and simulation approaches in order to reconstruct dynamic cellular processes from experimental data. The aim is to be able to make predictions

about plant growth and its relationship to biomass production with the help of this model. Understanding these processes and how they are controlled is gaining ever more importance because plants are increasingly being used as suppliers of sustainable resources.

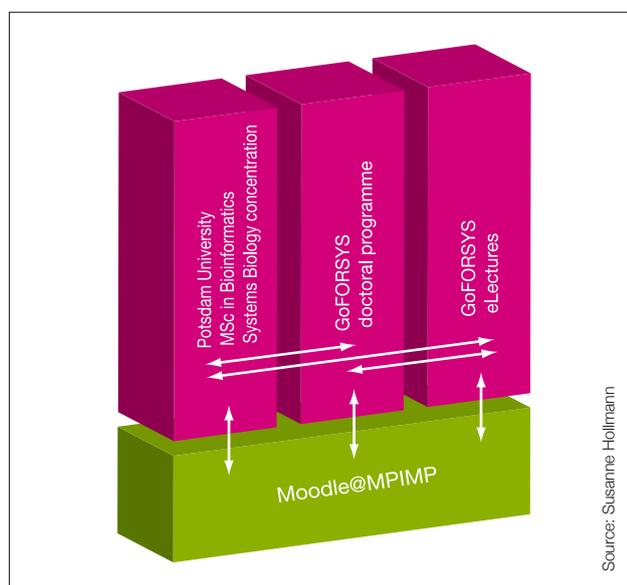
This research is based on an exchange between experiment and theory, and in particular, it requires young scientists who have been extremely well educated. The courses of study in this area that existed a few years ago were extremely subject-specific, but today emphasis is placed on an interdisciplinary approach. The barriers between scientific disciplines that were erected because one subject area does not understand how the others think are being overcome. The scientists in the GoFORSYS network have the primary aim of teaching young scientists an interdisciplinary approach and creating optimal learning conditions for them. That is why they have set up a Bioinformatics masters programme with Systems Biology as the major area of concentration, which also opens up an international perspective to young scientists.

Bridges between disciplines: the Bioinformatics Masters programme

The Masters programme combines life sciences content and computer sciences. Students learn to work in interdisciplinary teams. The course of study is available to people with Bachelor degrees in Computer Sciences as well as students with degrees in Life Sciences. 'Bridge modules' help students to close gaps in their knowledge of other subjects. In biology-orientated bridge modules, computer scientists learn the basics of molecular biology, cellular biology and biochemistry. Students with life sciences backgrounds will become familiar with operating systems and programming languages.

In later semesters, the focus is on bioinformatics. The modules in the first and second semesters are devoted to theoretical and applied aspects of bioinformatics. As opposed to courses of study that only focus on computer sciences, the curriculum in Potsdam aims to qualify students for working according to a systems biology

The three pillars of systems biology education in Potsdam



Source: Susanne Hollmann



approach. They become familiar with experimental systems biology, methods of genome research and the principles of metabolism. Aspects of selected signal transduction pathways also play a role. The main emphasis is on practical training in a laboratory setting; therefore in the third semester, the students form interdisciplinary working groups to complete a project that runs for several weeks. They complete the course of study with a thesis in the fourth semester.

The doctoral programme promotes soft skills and hard skills

The second pillar of the GoFORSYS network is an internationally orientated doctoral programme in the area of plant genomics and systems biology. Supported by the Max Planck Institute for Molecular Plant Physiology and the Mathematics/Natural Sciences department at Potsdam University, the programme supports doctoral candidates while they are obtaining their degree and collecting research results. In the process, they also acquire key qualifications for their professional careers.

As in the Masters programme, interdisciplinary teams supervise the candidates and their projects on a continuous basis. This takes place in the form of oral and written interim reports and team discussions, and with the assistance of presentations in the monthly GoFORSYS seminar. The scientific qualification programme features the greatest degree of practical work possible. In co-operation with International Max Planck Research School

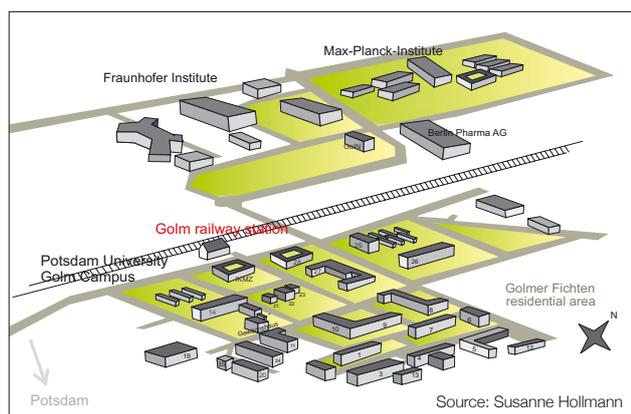
for Primary Metabolism and Plant Growth (IMPRS-PMPG) and Potsdam Graduate School (PoGS), courses on soft skills in the areas of communication, presentation, leadership and management are offered. The GoFORSYS doctoral programme also features a hard skills curriculum of internships, workshops and seminars that convey knowledge on the technology and methods of systems biology. Ring lectures on the basics and new research results in the areas of photosynthesis, plant metabolism, bioinformatics and modelling are an open component of the curriculum that goes beyond the borders of GoFORSYS.

Platform for eLearning and the exchange of information

Students and teachers both have access to a central, internet-based organisational and communications platform with user forums and a download area. Students also use the internet to register for courses and evaluate them. Discussion forums and chats facilitate communication between the doctoral candidates and their teachers. eLectures on selected systems biology topics are a special eLearning component.

In sum, the resulting synergy effects create specialised depth and interdisciplinary breadth in research and teaching. Potsdam University and the Max Planck institutes all agree that these research and educational activities will have to be sustained beyond the current five-year BMBF funding period in order to incorporate the unusually dense GoFORSYS network and knowledge base in future projects as well.

Potsdam University Golm campus: Productive proximity to extra-university research institutions



Dynamic region for research

The focus area of Plant Genomics and Systems Biology, in which basic research and applied aspects of plant biology are examined using algae, model plants and medicinal and industrial plants, was set up at Potsdam University. The area applies methods from bioinformatics and mathematical modelling. Another sign for the continuation of the GoFORSYS network is the filling of the post of Professor for Mathematical Modelling and Systems Biology. In addition, Potsdam University sponsored an initiative among the leading research institutions in the region that is unique in Germany. They have joined together to form a network called 'pearls - Potsdam Research Network'. The network includes nationally and internationally renowned scientific institutions, including



Bioinformatic masters programme: multi-disciplinary education with international perspectives (Source: Karla Fritze)

three Max Planck Society institutes, five members of the Helmholtz Association of German Research Centres, three Fraunhofer Society institutes and Hasso Plattner Institute for Software Systems Technology.

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Structured doctoral programme

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Fermenter for the cultivation of algae under a variety of lighting conditions (Source: J. Rupprecht)



cellular decisions

Key regulator in decision-making cascade of cells identified

By Marcel Schilling and Ursula Klingmüller

A cell constantly has to make decisions. Should it divide, change into another cell type or die? Signaling networks in which numerous proteins communicate with each other control cellular decisions (Fig. 3). If one of these proteins mutates, this has implications for the decision-making processes. One possible consequence is uncontrolled cell growth leading to cancer. A signaling network that plays a role in carcinogenesis is the MAP kinase cascade. One of its key proteins is the kinase ERK. Scientists at the German Cancer Research Center in Heidelberg and the University of Freiburg have now discovered how this key regulator works. A combination of modelling and experimentation led the scientists to success.

Kinases control the fate of cells

Human cells are constantly interacting with their surroundings. In the process, growth factors or hormones bind to receptors on the surface of cells. Following this, the intracellular part of the receptor changes its shape allowing the recruitment and activation of signal transmitting proteins. Important components of signal transmission in the cell are the kinases. These proteins are enzymes that change the properties of other proteins by means of phosphorylation, thereby enabling for example the activation of further kinases. As a result of kinase cascades that involve a large number of different proteins, signals can be transmitted from the surface to the nucleus of the cell that then regulate the activity of target genes. A prime example of this type of cascade

is the MAP kinase cascade, which consists of the RAF, MEK und ERK kinases. Information that is encoded by activated receptors on the cell surface is processed by these three serially connected kinases and linked to cellular decisions. The components of this cascade play a major role in a large number of diseases. Hyper-activation of the ERK kinase, for example, is observed in a large number of cancers and is frequently associated with cancers that are difficult to treat.

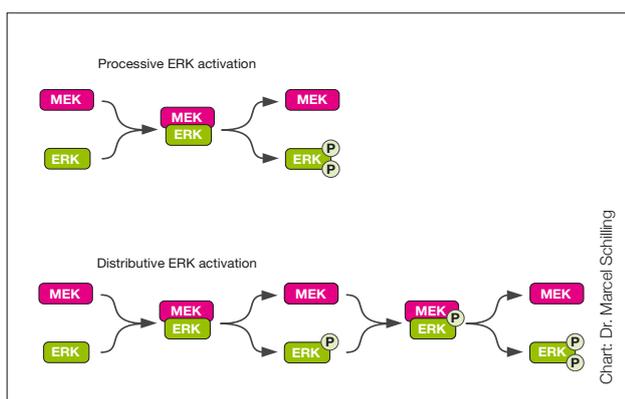
Although the components and interactions of this network are known, we still know little about information processing and how the activation of signaling molecules is converted into cellular decisions. If the mechanisms how signals are processed were better understood, it might be possible to identify new target proteins for systematic medical treatment.

Key regulator of the decision-making cascade: the ERK kinase

As part of the project outlined, the ERK kinase (short for Extra-cellular Regulated Kinase) was investigated in detail. Two closely related variants (isoforms) of ERK – ERK1 and ERK2 – occur in mammalian cells. However, the role that these two isoforms play was largely unknown. Several experiments indicated that ERK1 and ERK2 are interchangeable, on the other hand mice that lack the ERK1 protein are viable, whereas a lack of ERK2 is lethal.

As connections in biological signaling networks are non-linear and highly complex, only a systems biology approach that takes

Fig. 1: ERK activation



Processive ERK activation (above) needs only one binding step, whereas distributive ERK activation (below) requires two binding steps. With a processive mechanism signals can be relayed faster, whereas a distributive mechanism leads to an amplification of the signal.



Dr. Marcel Schilling investigates signaling networks at the German Cancer Research Center by combining experiments and computer models.

as many factors as possible into account allows to investigate the dynamic interplay of signaling pathway components quantitatively and to decipher their properties. One molecule is not just considered in isolation; the entire network is analysed with all of its interactions and dynamic processes. To establish a computer model, a signaling cascade is translated into mathematical equations. The calibrated model enables computer simulations to simulate the influence of individual components and predict changes. One initial unresolved issue concerned the activation mechanism of the ERK kinase, which includes phosphorylation of the protein at two residues. There had previously been two theories as to how this might occur: (i) In a processive, or one-step mechanism, the kinase MEK binds to ERK and phosphorylates both residues in a single step so that the signal can be relayed very fast or (ii) in a distributive, two-step mechanism, the kinase MEK binds to ERK and phosphorylates first one residue, whereupon the complex disintegrates and has to be recreated to phosphorylate the second residue (Fig. 1). In distributive ERK activation MEK must twice form a complex with ERK so that in this scenario the MEK concentration has a greater influence on the activation and might facilitate signal amplification between MEK and ERK. Test-tube experiments of isolated proteins indicated a distributive mechanism; however, in living cells additional components that are not present in the test tube might stabilise the complex and thereby promote a faster, processive mechanism.

Computer promotes experiment

To decipher which of the two mechanisms is used in mammalian cells we established a computer model of the ERK signaling network that is activated by the hormone erythropoietin (Epo) [1]. Epo is an essential factor in the formation of red blood cells because it ensures the proliferation and specialisation of the progenitor cells. To make the model as realistic as possible we generated by biochemical experiments time-resolved data of erythroid progenitor cells after treatment with Epo and adjusted the model's parameters to this data. Freshly isolated primary cells were used for these experiments because signaling networks ex-

ist rather unchanged in these cells in contrast to the frequently used cell lines that are propagated over long periods in culture and are genetically unstable [2]. A quality criterion for our model was that it was able to reproduce not only the temporal changes of activated signaling proteins but also the effect of different Epo concentrations.

To clarify the underlying mechanism of ERK activation, we set up two alternative models in accordance with the possible activation mechanisms described above. Interestingly, only the model based on the distributive two-step mechanism was able to describe the data observed in the experiment (Fig. 2). Model predictions showed that the mechanisms differ in their amounts of single and double-phosphorylated ERK. Accordingly, the two computer models were used to predict the concentrations of unphosphorylated, single and double-phosphorylated ERK after treatment with Epo. Subsequently, the actual concentrations of these molecules were determined by using a newly developed quantitative mass spectrometry method. The experimental results were only in accordance with the predictions for the distributive model and not with those for the processive model. Thus, we were thereby able to demonstrate that in primary mammalian cells ERK activation is mediated by a distributive mechanism.

How cell proliferation is restrained

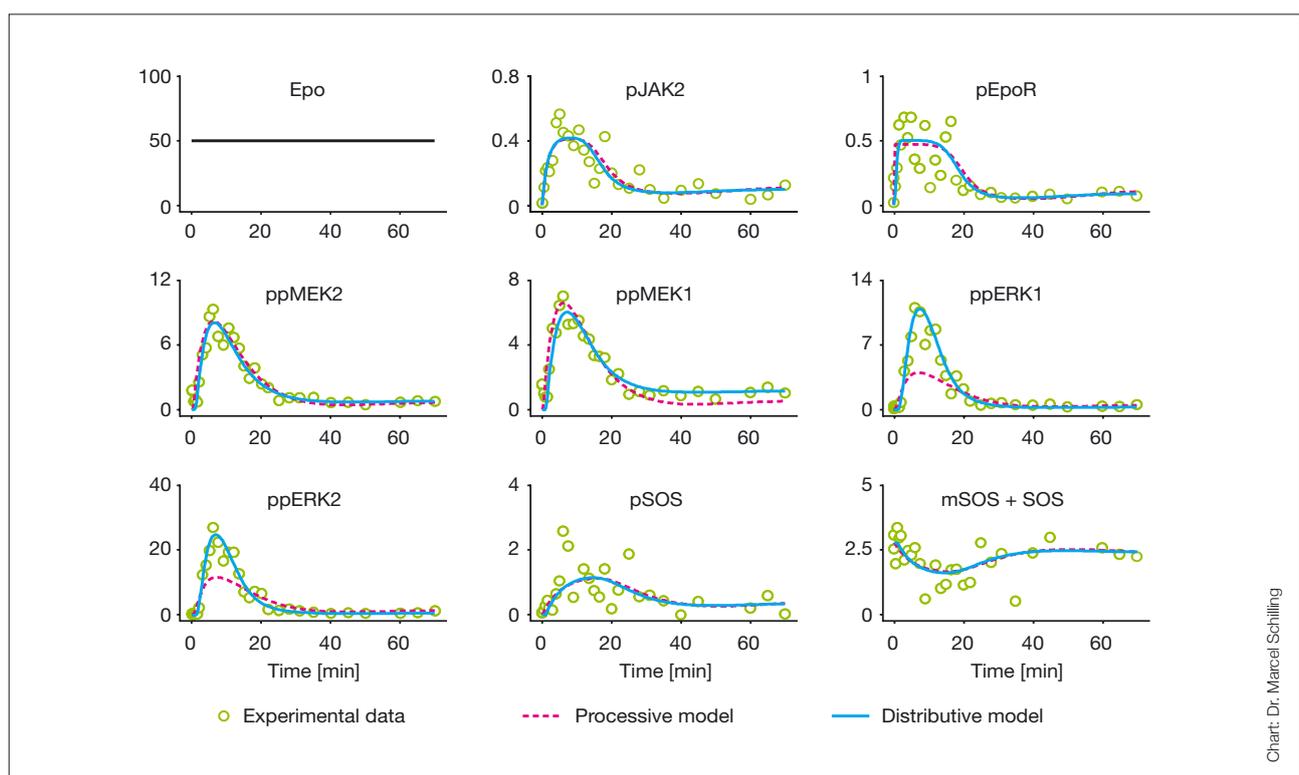
In addition, we were able to use the computer model to analyse further details of information processing in this signaling network. We calculated the concentrations of activated molecules at every point in the network and showed a high level of signal amplification mediated by the distributive activation mechanism of ERK. The computer simulations also provided further unexpected insights into the wiring of the signaling network and predicted that an increase in the ERK1 concentration might increase ERK1 phosphorylation but at the same time lead to a reduction in ERK2 phosphorylation. We were able to experimentally confirm this unexpected property by artificially increasing the ERK1 or ERK2 concentration.

To link signal activation with cellular decisions, the degree of ERK1 and ERK2 activation was calculated for different Epo concentrations. For this we determined proliferation of erythroid progenitor cells with the addition of different amounts of Epo. To dissect the contribution of the two ERK isoforms the amount of ERK1 or ERK2 in the cells was artificially increased. We were able to demonstrate that an additional increase in ERK2 and especially in ERK1 inhibits cell proliferation even though Epo actually supports cell division. The inclusion of extensive experimental data enabled us to determine a mathematical function that maps the connection between signal response and cell proliferation in detail. Analysis of the individual contributions made by activated ERK1 and ERK2 revealed that ERK1 hyperactivation reduces proliferation whereas ERK2 intensifies cell proliferation to a certain extent. This means that high ERK1 phosphorylation rates prevent cell proliferation. This could be a mechanism to combat uncontrolled cell proliferation.

To sum up, we demonstrated by combining experimental analyses and computer models that within the MAP kinase cascade the ERK kinase influences cellular decisions in a dose-dependent and isoform-specific way. The distributive ERK activation mechanism reproduced in the model and demonstrated in experiments facilitates strong signal amplification within the signaling network. The results achieved are of great medical relevance because they make it easier to understand how ERK activation observed in many kinds of cancer affects cellular decisions. Determining the specific functions of the isoforms ERK1 and ERK2 lays an important foundation for system-oriented drug research and thereby opens up new ways to treat cancer.

Fig. 2: Computer model predicts change in concentrations observed

Changes in concentration of the most important components in the signaling network investigated. The changes observed in experiments (green circles) cannot be described using the processive model (simulation in red) but only by the distributive model (simulation in blue).



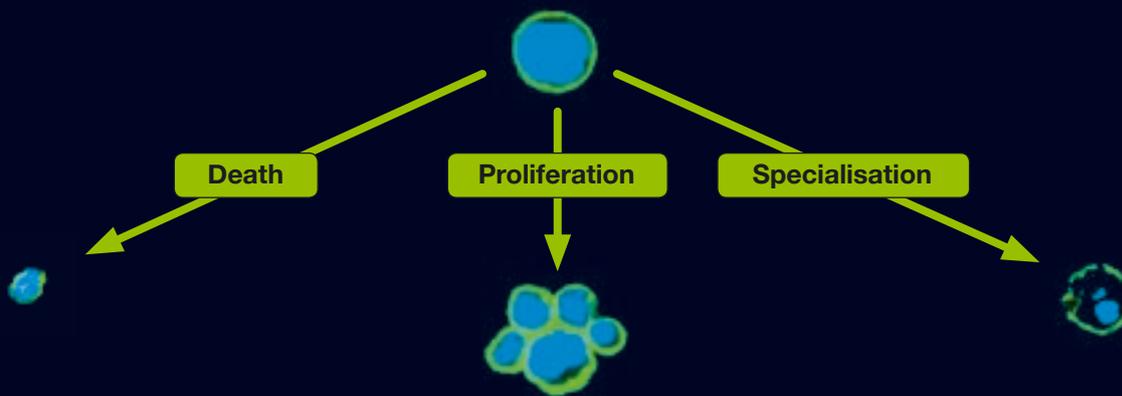


Fig. 3: Complex signaling networks control cellular decisions such as death, proliferation or specialisation.

The research project in brief:

Project name: SBCancer: Systems Biology of Signaling in Cancer
 The Systems Biology of Signaling in Cancer (SBCancer) research network is part of the Helmholtz Alliance on Systems Biology, an initiative to promote systems biology in Germany financed by the Helmholtz Association's Initiative and Networking Fund with which Helmholtz Centres, universities and other partners are associated.

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expert in oscillations and networks

A portrait of Ursula Kummer

By Thomas Früh

When Ursula Kummer took up her professorship in March 2007, she shared with her surroundings the pleasure of a fresh start. She and her workgroup were not the only ones unpacking their boxes. Scientists were busy moving into the other rooms too. Professor Kummer's new domain was the BioQuant Center for Quantitative Analysis of Molecular and Cellular Biosystems in Heidelberg, which had just opened. Kummer, now 43, whose Modelling of Biological Processes division was made possible by a professorship endowed by the Klaus Tschira Foundation, found ideal conditions at BioQuant. Bioscientists who approach their subject in laboratory experiments work under one roof alongside experts in theory and modelling. The working tool of preference in Ursula Kummer's team is the computer. It helps her to shed light on the jungle of complexity that surrounds life processes.

Even in complexity there are patterns – oscillations, for example. At the University of Oregon her interest in the subject was stimulated by a lecture given by Anatol Zhabotinsky, one of the two men who gave their name to the Belousov-Zhabotinsky oscillator reaction. As part of her degree and PhD in Tübingen, she went on to investigate processes in cells that oscillate.

The dynamics are what matters

At the non-profit EML Research Institute in Heidelberg, where she worked from 1998 to 2007, first as a post-doc, then as a group leader, she analysed how calcium concentrations in cells oscillate. She found out how oscillation frequency and patterns can transport information. This kind of signal transmission enables cells to use the same messenger substance for different messages. It also means that the mere statement of a concentration level

reveals little about the effect of a substance. "Thinking in linear causal chains along the lines of 'substance A increases B' is not enough. We need to know how dynamically A changes," Kummer concludes.

Her interdisciplinary approach would also appear to be the result of oscillation. She showed a clear and early interest in science. As a primary school pupil she collected caterpillars because she wanted to know which butterflies they became. But when it came to choosing a subject to study, she was undecided. "I alternated between biochemistry and physics for a long time," Kummer says. She solved the dilemma by studying both. Her knowledge of the two disciplines, one of which was more empirical and experimental and the other more theoretical and mathematical, today makes it easy for her to make the lateral connections that her work in systems biology requires.

Software as a research tool

There is no lack of lateral connections in signal and metabolic pathways. To clarify them, her group at the EML joined forces with Pedro Mendes of the Virginia Bioinformatics Institute to develop the software package Complex Pathway Simulator, or COPASI for short. Its special characteristic is that a single solution provides a wide range of methods to analyse, model and simulate networked biochemical reactions.

In developing the software Kummer and Mendes attached great importance to user-friendliness. "Bioscientists," she says, "today use databases as a matter of course. We want computer modelling to become an everyday tool too." COPASI is used not only



Prof. Dr. Ursula Kummer heads the Modelling of Biological Processes Research Group at the Heidelberg BioQuant Center.

in basic research but now also by the pharmaceutical industry. “Whether a drug will work on one person and not on another cannot usually be explained by individual factors. Connections and reciprocal effects are important instead,” the systems biologist says.

“A project that is one of a kind in the world”

As a programme director, Kummer prepared from July 2009 to March 2010 for the launch of Virtual Liver, the successor programme to HepatoSys in which around 70 teams of medics and bioscientists working experimentally and modelling all over Germany are networked. The research network aims to explain liver diseases and the liver’s medication metabolism.

“The Virtual Liver project is one of a kind in the world. The results submitted by individual research teams are merged into a joint model that maps the networked signal and metabolic pathways of the liver,” Kummer says. The interdisciplinary organisation of research corresponds to her network-like research subject. “Cooperation is indispensable for scientific progress. On every subject our modellers have experimental teams as partners,” she adds. At the Heidelberg Center for Modelling and Simulation in the Biosciences (BIOMS) which Ursula Kummer coordinates jointly with Willi Jäger, this kind of cooperation has been practised successfully since 2004. And just as the systems biologist takes her interdisciplinary research area forwards by means of a wide range of cooperation projects, she also backs progress in another area. “In my group the proportion of women

is 50%. At BioQuant, scientists with children can work flexibly and part-time.” This is how easy she finds it to reconcile a family and a career. For Kummer, life also exists beyond the world of research: “To remain creative I need to strike a balance, and I find one in the family and in music.”

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ERASysBio+

A wide-ranging systems biology research area

By Bernhard Gilleßen

Expanding horizons is one of the objectives of ERA-SysBio, an initiative to develop the European Research Area for Systems Biology. ERASysBio aims at making systems biology in the ERA internationally competitive by means of strategic activities. Three transnational initiatives, SysMO, SysMO2 and ERASysBio+, have so far been put in place together with European partners. While SysMO focuses on systems biology of microorganisms, in ERASysBio+ transnational research projects in three subject areas – biotechnology, biomedicine and agri-food production – were able to apply for funding. The prerequisite for funding was a systems biology approach linking experiments and models with the aim of making forecasts about systems. Since March 2010, 16 transnational joint projects have implemented this approach in projects involving 85 research teams from 14 European and non-European countries.

Ministries and institutions from ten EU member-states provide funding. Over the next three years they are investing €24 million in systems biology research, including €5.5 million EU funds. The German Federal Ministry of Education and Research (BMBF) is providing about €7.6 million, which is one of the largest contributions in Europe. Project Management Jülich is coordinating the initiative on behalf of the BMBF. German scientists are involved in ten of the 16 network projects and German group leaders are coordinating three of them.

ERASysBio+ is one of the first ERA-NET Plus measures. As part of the ERA-NET framework programme its purpose is to contribute to the coordination of national research programmes and to create a European Research Area. Uniformity is not the objective; the aim is to coordinate a wide range of activities and consequently foster competitive abilities. The research topics which ERASysBio+ is investigating are also characterised by diversity. They range from human, via animal and plant cells to organs and organisms. Infections, cancers and age-related diseases are investigated, as well as behaviour and embryo development.

The ERASysBio+ projects

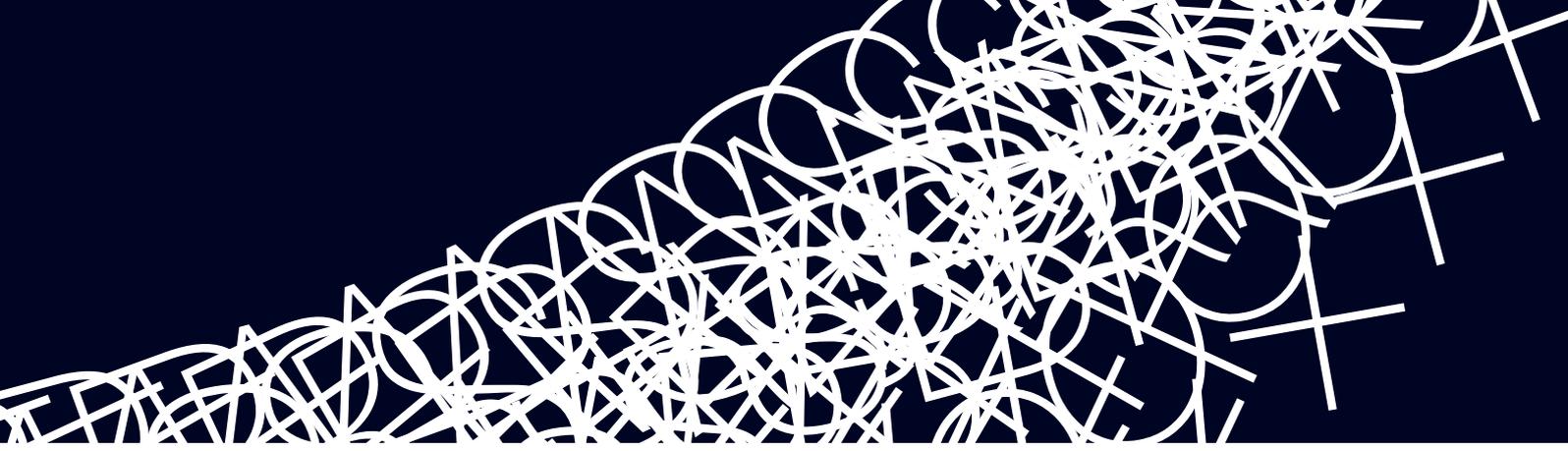
How cells respond to stress or attack

How do cells respond to harmful environmental influences such as chemical or physical stress? How do they defend themselves against pathogens? Signal transduction always precedes the cell's response. First the information indicating stress or an attack has to reach the cell's control centre, the nucleus. There the genes' activity patterns change, which subsequently has an effect on the protein configuration, the metabolism and the cell's structures. These responses are far from being straight process chains, instead they are complex and networked. If you want an insight into how this works, you need to bring a computer in the lab to combine experimentation and modelling.

This is the way a research network headed by Prof. Judith Klein-Seetharaman of the *Forschungszentrum Jülich* is analysing how animal and vegetable cells **react to a salmonella attack**. Scientists led by Dr. Andrzej M. Kierzek of the University of Surrey in Guildford, UK, are taking a closer look at different cell types in the human immune system and they are comparing their **response to the tuberculosis pathogen**.

An international research project coordinated by Prof. Andrew Cossins of the University of Liverpool is also focusing on differences and common features. It is examining 200 genetic variants of the model nematode *Caenorhabditis elegans* which is exposed to **controlled stress treatment** to shed light on the connection between individual predisposition and complex illnesses.

For the human liver, stress may consist of fatty food or drugs. Depending on the stress level, the liver can develop certain symptoms of illness with different prognoses. This is where research scientists led by Dr. James Adjaye of the Berlin Max Planck Institute of Molecular Genetics approach the subject. They are analysing liver cells taken from different groups of patients to develop a model for forecasting the **liver's stress reaction**.



How cells specialise

In organisms there is a differentiation between organs and tissues consisting of specialised cells. These “specialists” in, say, immune defence or transporting oxygen develop from less specialised cells and ultimately from stem cells which are not or only partly determined in terms of their function. In medical terms it is extremely important how specialised tissue originates and abnormally degenerates. **Bone or cartilage** which is damaged by osteoporosis or arthritis could, for example, be reconstituted by stem cells. Gene regulatory mechanisms which play a part in this process are under investigation by a team headed by Prof. Joop von Zoelen of the University of Nijmegen.

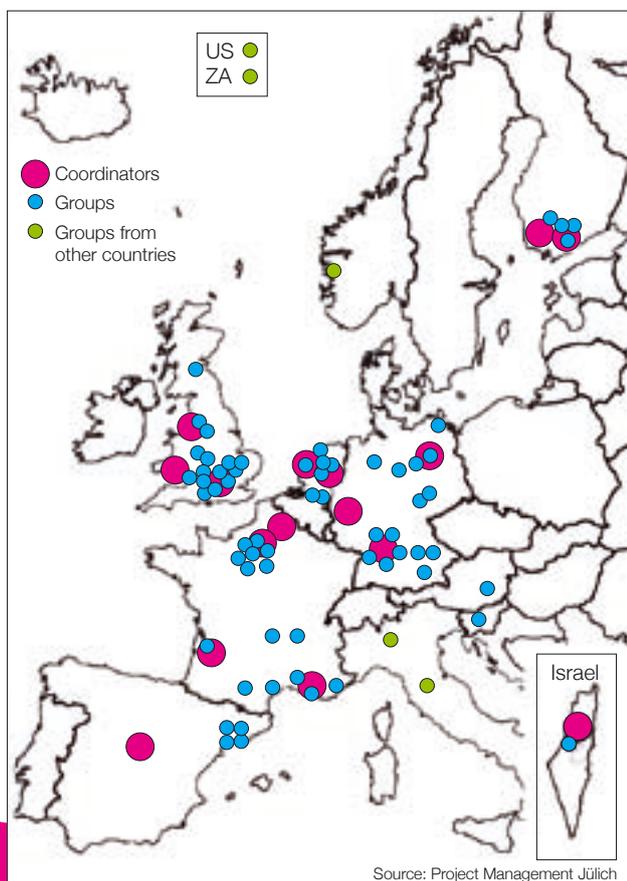
A team led by Prof. Riitta Lahesmaa of the University of Turku, Finland, is investigating how certain T lymphocytes generate the

newly discovered cell type Th17. These **immune defence** cells participate in causing rheumatoid arthritis and possibly multiple sclerosis too.

The heart’s development is also a result of cell differentiation. Using the fruit fly *Drosophila* as a model system, research scientists headed by Dr. Laurent Perrin of the University of Marseille are gaining new insights into healthy and pathological developments of the human heart. The gene regulatory processes which control cardiac developments are conserved from fruit flies to mammals.

Plant stem cells are located at the tips of stems and roots in the apical meristems. Throughout a tree’s life span they regularly produce new tissues and organs, such as leaves. A project undertaken by Prof. James A.H. Murray of the University of Cardiff is investigating how the hormones auxin and cytokinin control the development of stem apical meristems.

Distribution of ERASysBio+ research groups



A tomato’s taste and its content of nutrients is a result of the complex interaction between genes and environmental factors are operative while the tomato ripens. To clarify this interaction, experts in fruit biology, ecophysiology and theoretical biology headed by Dr. Yves Gibon of the INRA Institute, Bordeaux, are creating a “virtual tomato”.

Cancer as a result of a wrong cellular decision

Receptor molecules in the cell nucleus – **nuclear receptors** – are **nerve centres of cellular development**. They pave the way for organ development and tissue growth, maintenance and renewal. They also have effects on malfunctions such as certain forms of breast and prostate cancer. A research network led by Dr. Sampsa Hautaniemi is investigating how nuclear receptors and other factors control gene activity.

Hepatocellular carcinoma is an especially dangerous form of cancer, which develops from liver cells, because chemotherapy is unable to trigger programmed cell death in the carcinoma. A project headed by Prof. Michael Boutros of Heidelberg University aims to identify the signalling pathways that determine when a liver cell “commits suicide”.

The cell cycle with its key events such as differentiation, growth, cell division and death is linked to the **rhythm of the inner, circadian clock**. Research scientists headed by Dr. Francis Levi of the Paul Brousse Hospital in Villejuif, near Paris, are attempting to find out how the two rhythms interact for healthy and malignant cells. They focus on the influence on cell reproduction and the sensitivity to changes in genetic make-up.

A question of architecture

The sequence of building blocks in the human genome has meanwhile been explained. In contrast, the interplay between the **genome's three-dimensional structure** and its function is still unresolved. In spite of an increasing indication that the complex spatial architecture in and between the chromosomes exerts a considerable influence on the regulation of gene activity, we can still not predict how genes which are "transplanted" into other chromosomal areas will behave. Prof. Tobias Knoch of the Erasmus Medical Center, Rotterdam, and his partners are trying to uncover the links between structure and function. To achieve this, they are linking theory and experiment in a unique approach.

Of neurons and muscles

Neuronal information processing depends on the interconnection of the nerve cells, but also on processes within the cells. Prof. Kobi Rosenblum of the University of Haifa and his partners are analysing how **protein synthesis in the synapses** responds to electrical activity patterns. Thereby they hope to gain a better understanding of the phenomena of memory and learning.

Deciding between two courses of action is the research topic of a network led by Prof. Gonzalo de Polavieja of the Cajal Institute in Madrid. The researchers are exposing zebrafish larvae to ambivalent stimuli and are then recording their activity in neuronal networks as well as the resulting behaviour. Their aim is to clarify the influence of neuronal processes on decision-making.

The smooth musculature of the uterus generates electric potentials that can be derived from a pregnant woman's abdomen. This type of electromyogram, the electrohysterogram, would

appear to be a highly promising means of determining the onset of contractions at an early stage thereby preventing pre-term births. Therefore, a team headed by Prof. Catherine Marque of the Technical University of Compiègne is investigating how signals measured outside the uterus are linked to muscular and hormonal activities inside the uterus. The research scientists intend to make all their data and findings readily accessible and to develop clinical standards for electromyography of the uterus.

Representatives of all networks, reviewers and sponsors met at the ERASysBio+ kick-off in Paris on 17 and 18 May 2010. All of the projects were presented in a festive frame and the importance of the initiative for the European Research Area was underscored. For further information, images and contacts for individual projects please visit

www.erasysbio.net

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new insights into the auto-regulation of mammalian cells

Modelling delivers in-depth insights into how mitochondria work

By Uwe Jandt, Oscar Platas Barradas, Ralf Pörtner, Eva Schröder, Thomas Noll
Volker Sandig and An-Ping Zeng

Central nodes in the energy metabolism of mitochondria, the cell's powerhouses, are subject to sophisticated regulation. An intelligent combination of modelling techniques and experiments makes these regulatory mechanisms easier to understand. Recent advances in this area are due to a successful university and cross-institutional cooperation funded by the German federal FORSYS-Partner research initiative.

Our ability to collect measurement data from living cells has increased dramatically in recent years. Not only the genome but also protein sets and metabolic pathways are becoming increasingly accessible for analytical methods. Our picture of the interplay of genes, proteins and metabolites, their auto-regulation and events in the cell cycle is growing more complete step by step. New or further developed techniques such as flow cytometry, also known as FACS or Fluorescence Activated Cell Sorting, are enabling us to analyse the distribution of properties in large and heterogeneous cell populations. Confocal and STED microscopy [Schmidt et al., 2009] also provide insights into the spatio-temporal distribution of proteins in living cells and cell compartments down to a resolution of a few dozen nanometres.

Putting this plethora of analytical and data processing techniques to optimal use is a challenging task that requires cross-institutional collaboration. A case in point is the SysLogics collaborative project that is funded as part of the FORSYS-Partner systems biology initiative by the German Federal Ministry of Education and Research (BMBF). The project partners – four universities, two large-scale research institutions and a biotechnology company – aim to characterise a newly developed human production cell line comprehensively and take forward understanding of the auto-regulation of central cell processes.

Guiding laboratory experiments with the computer

For this purpose, it is mandatory to evaluate vast amounts of raw process and analysis data. This requires computer-based mathematical models in order to predict the behavior of cells cultivated in a bioreactor under defined conditions and to precisely guide subsequent experiments. As eukaryotic cells are extraordinarily complex, modelling must be limited to precisely defined partial aspects such as, for example, simulation of proteins at the atomic level (*molecular dynamics*) or, on a larger scale, intracellular transportation processes, protein secretion, the cellular cycle or regulatory processes at the genome level.

A decisive role in the auto-regulation of eukaryotic cells is played by the pyruvate metabolism, which represents a central node in the energy metabolism of mitochondria. This central metabolic pathway is closely linked to processes that control the cellular cycle, cell growth and programmed cell death, or apoptosis.

A finely balanced interplay

Cell division and cell differentiation are only possible by means of precisely balanced interactions between the central metabolism and the cell cycle. The pyruvate metabolism is subject to a network of competing and cooperating regulators. A loss of balance in this area can trigger typical civilisation diseases such as diabetes or cancer. However, quantitative comprehension of regulators and the influence of cellular and mitochondrial morphology and intracellular transportation mechanisms is still in its early stages, with the result that intensive research is still needed.

The pyruvate metabolism in the mitochondria is initiated by the conversion of pyruvate into acetyl-CoA, in the course of which CO₂ is released (Fig. 1). The catalytic enzyme complex required for this reaction, the pyruvate dehydrogenase complex or PDC, is located in the intramitochondrial matrix and consists of three different isoenzyme types, each in up to 60 copies, several co-factors and at least four kinase isoenzymes and two phosphatase isoenzymes for regulation. The resulting molecular mass of nearly ten megadaltons makes the PDC one of the largest biological molecular complexes.

Regulation of this complex, its spatiotemporal dynamics and the interplay of individual isoenzymes is the subject of a model developed by scientists headed by An-Ping Zeng at the Hamburg University of Technology's Institute of Bioprocess and Biosystems Engineering. This model maps the enzyme kinetics of four PDC isoenzymes and a total of six isoenzymes of the regulatory kinases and phosphatases along with various co-factors in a system of non-linear differential equations [Zeng al., 2002]. Non-linear dynamics such as oscillations and multiplicities were predicted on the basis of previous model versions. The model is currently being adjusted to map the specifics of the newly established human neuronal cell line AGE1.HN and the spatial distribution (Fig. 2) and internal structure of the mitochondria.

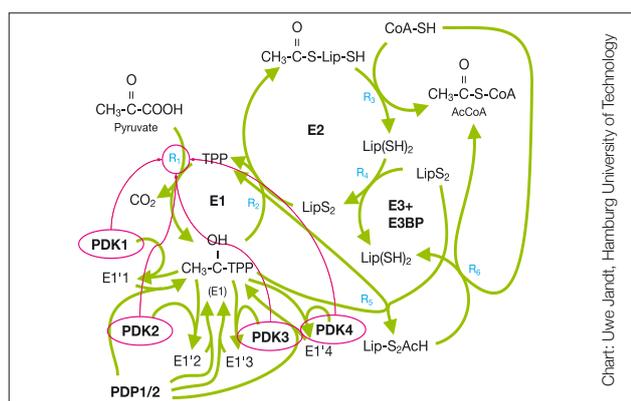
Feedback between experiment and model

With the assistance of the dynamic model the sensitivity of pyruvate conversion in the mitochondria compared with concentrations of individual regulatory kinase isoenzymes can be estimated in advance. This is of special interest particularly because we, together with ProBioGen AG and Thomas Noll's team at the University of Bielefeld, were able to prove the existence of the PDH kinase isoenzyme 1, or PDK1 for short, in the newly developed cell line. The consequences for regulation of this cell line's pyruvate metabolism must now be clarified.

With the aid of the model we are now specifying the boundary conditions for the experiments, and depending on the reaction of the kinase phosphatase regulatory system to the variation of external conditions, such as glucose and insulin impulses, the model will be extended accordingly.

A high cell density is needed to achieve reliable results, as is comprehensive control of process conditions. This is achieved by means of a dialysis process for cell cultures (Fig. 3) that was established at the Hamburg University of Technology's Institute of Bioprocess and Biosystems Engineering. During cultivation there is a continuous interchange of substrate and metabolic products between the internal cultivation chamber and the external media chamber. In this way, either constant media conditions or impulses can be realised as required.

Fig. 1: Schematic description of the main reactions of the pyruvate dehydrogenase complex (PDC)



Main reactions of the pyruvate dehydrogenase complex (PDC) and the regulating kinase and phosphatase isoenzymes in the mitochondria.

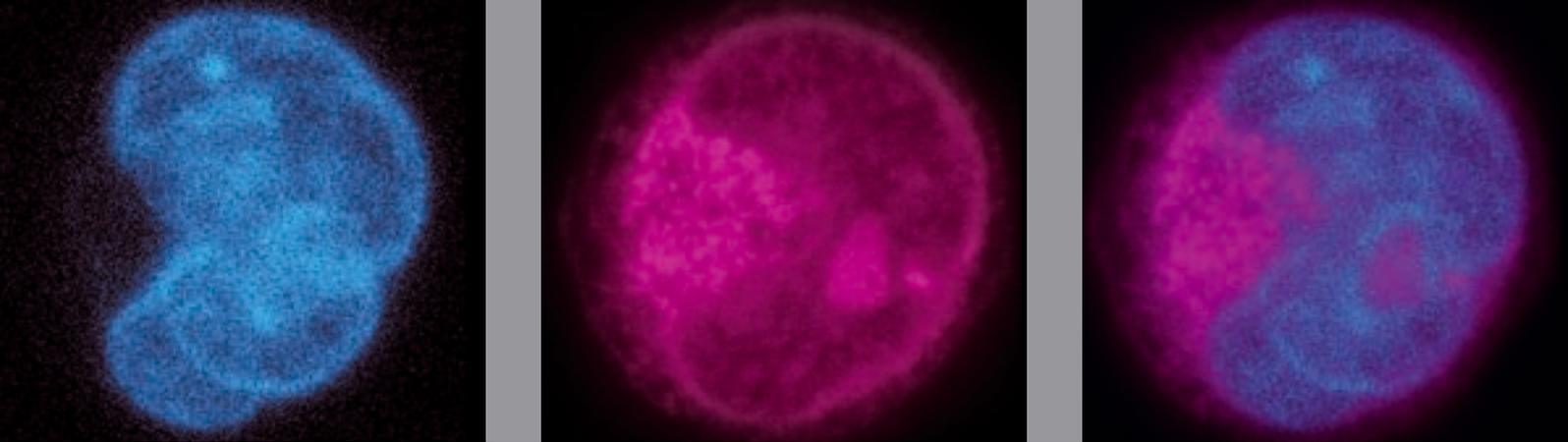


Fig. 2: Confocal microscopic images of AGE1.HN cells: (a) Dyed nucleus (DAPI, dyed blue), (b) dyed mitochondria (Invitrogen Mitotracker® dyed red) and (c) their overlay. The spatial distribution of the mitochondria is of particular interest for modelling because it is subject to a dynamic variations and exerts a significant influence on mitochondrial activity and regulation. (Photo: Maria Buchsteiner, Uwe Jandt, Oscar Platas Barradas, Hamburg University of Technology)

In further research we are going to clarify how metabolic products and external factors influence the kinase and phosphatase isoenzymes. The focal point of interest is the influence of cell cycle regulation. The model is also to be extended to include upstream and downstream processes of the central metabolism, especially glycolysis and the citrate cycle.

The model is being developed continuously and is to become an important tool for systems biology. It can support optimisation of cultivation and development of cell lines and, in addition, contribute toward clarification of the complex regulatory networks in the cells.

The research project in brief:

Project name: Systems Biology of Cell Culture for Biologics – SysLogics

A network project as part of the BMBF initiative FORSYS-Partner that aims to further strengthen the potential of systems biology in Germany by incorporating external expertise into existing FORSYS centres by means of FORSYS cooperation arrangements.

The project cooperates with the Magdeburg FORSYS Centre (MaCS).

Associated partners: Hamburg University of Technology, Institute for Bioprocess and Biosystems Engineering; An-Ping Zeng, Ralf Pörtner; Bielefeld University, Institute for Cell Culture Tech-

nology; Thomas Noll; Bioinformatics Resource Facility: Alexander Goesmann; Saarland University, Biochemical Engineering; Elmar Heinzle; University of Hannover, Institute of Technical Chemistry: Thomas Scheper; Max Planck Institute for Dynamics of Complex Technical Systems Magdeburg; Udo Reichl; Helmholtz Centre for Infection Research, Division of Molecular Biotechnology: Hansjörg Hauser, Dagmar Wirth; ProBioGen AG Berlin: Volker Sandig

www.forsys.net

www.forsyspartnerssyslogics.de

www.tu-harburg.de

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Rose T., Winkler K., Brundke E., Jordan I., Sandig V. (2005). Alternative Strategies and new cell lines for high-level production of biopharmaceuticals. In: Knäblein J., editor. *Modern Biopharmaceuticals*: Wiley-VCH. p 761-777.

Patent application WO2007054516A1; Sandig V., Jordan I., Brundke E. Productivity augmenting protein factors, novel cell lines and uses thereof

Fig. 3: Dialysis reactor for cultivating AGE1.HN cells



Cells are cultivated in the internal chamber and the medium in the external chamber can be renewed or exchanged as required.

Photo: Oscar Platas Barradas, Hamburg University of Technology

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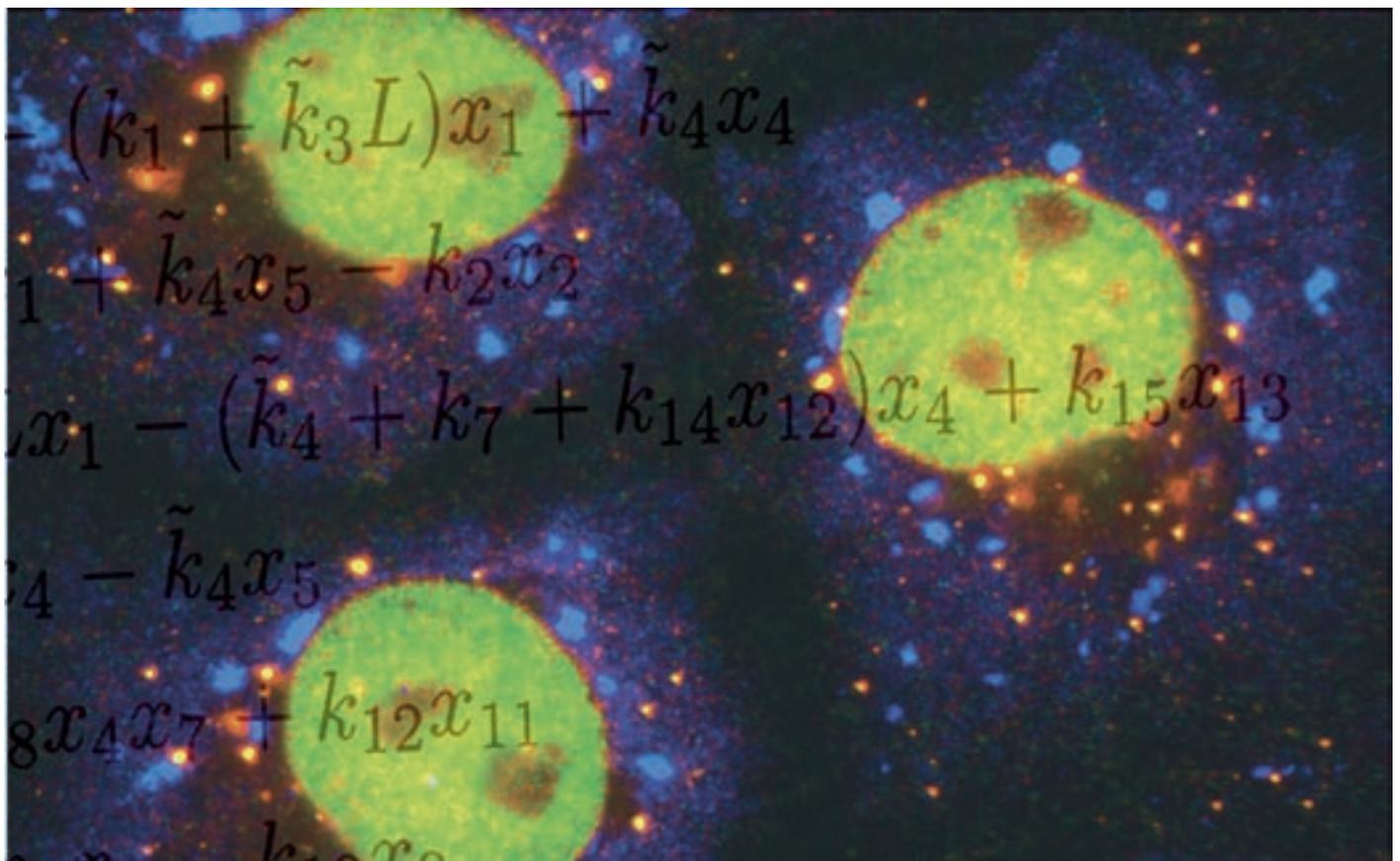
PRESENTING THE HELMHOLTZ ALLIANCE ON SYSTEMS BIOLOGY

Systems biology is a multidisciplinary research area in which mathematicians, physicists, biologists and engineers work together to decipher quantitative connections in cells and organisms. To carry out research into complex mechanisms that are decisive for certain diseases, six Helmholtz centres and twelve other research institutions have joined forces to set up the Helmholtz Alliance on Systems Biology.

The Helmholtz Association is providing the Alliance with a total of € 49 million in funding by 2012, with the President contributing half of this amount from the Initiative and Networking Fund resources. Together with its partners, the Helmholtz Association is making an important contribution toward major and pressing healthcare research issues. It is using the financial resources earmarked in the Joint Initiative for Research and Innovation to continue and develop strategic measures, to extend existing instruments qualitatively and quantitatively and to develop, test and establish new instruments.

The six Helmholtz centres:

- German Cancer Research Center (DKFZ), Heidelberg
- Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch
- Helmholtz Centre for Environmental Research – UFZ, Leipzig
- Helmholtz Zentrum München – German Research Center for Environmental Health
- Forschungszentrum Jülich
- Helmholtz Centre for Infection Research, Braunschweig



Biological experiment and mathematical modelling

Photo: German Cancer Research Center

THE SIX HELMHOLTZ CENTRES AND THEIR PROJECTS

German Cancer Research Center – The SBCancer (Systems Biology of Signalling in Cancer) Network

The main focus of the SBCancer network is the cellular signalling pathways that play an important role in the decision between cell proliferation, cell differentiation and cell death. Changes in these signalling pathways and the associated gene regulation networks can influence these decisions and thereby lead to uncontrolled growth, i.e. tumours. Understanding how tumour cells interact with surrounding tissue also plays an important part. Experimental findings are tested in simulation models to find out how molecular signalling pathways that lead to cancer can be switched-off most effectively. This model-based understanding of cancer signals is used in pilot projects for initial translational research applications.

THE CENTRE:

The German Cancer Research Center is the largest biomedical research facility in Germany. More than 2,000 employees, including 580 scientists, are involved in researching the mechanisms of the origins of cancer and recording cancer risk factors.

Max Delbrück Center for Molecular Medicine – The MDC Systems Biology Network (MSBN)

The MSBN network focuses on Alzheimer's disease and the NF κ B signalling pathway and its significance for cancer and neurodegenerative diseases. Both are characterised by lengthy asymptomatic phases before the onset of the disease. Regulatory mechanisms first influence compensation and later the course of the disease; they are being investigated using a comprehensive systems biology strategy.

THE CENTRE:

The Max Delbrück Center for Molecular Medicine in Berlin with its 1,400 employees combines basic research in molecular biology and clinical research with the aim of developing new methods to diagnose and treat serious diseases. State-of-the-art molecular biology methods are used for research in cardiovascular and metabolic diseases, cancer research and the function and dysfunction of the nervous system. To ensure that findings benefit patients as quickly as possible, MDC research scientists cooperate closely with clinicians at Charité – Universitätsmedizin Berlin.



Photo: Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch

Helmholtz Centre for Environmental Research – UFZ – Development of Predictive Models

The main focus of the UFZ's systems biology network is on investigating cell reactions to chemical stress factors from a systemic perspective. Cellular reactions to polycyclic aromatic hydrocarbons are investigated because these chemicals are known to trigger a large number of toxic effects.



Microbial ground water decontamination

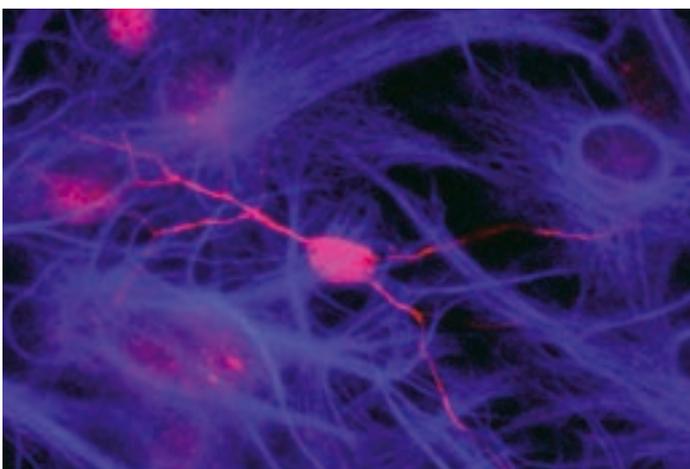
Photo: Helmholtz Centre for Environmental Research – UFZ

THE CENTRE:

At the Helmholtz Centre for Environmental Research – UFZ, 950 employees work on the prevention or reduction of dangers and risks to humans and nature. The UFZ evaluates and processes research findings so that they can be implemented in the decision-making processes of government and business and transferred to regions with similar problems. The UFZ, for instance, advises government agencies on the clean-up, ecological redesign and management of ecosystems and business on developing environmental technologies and environmentally friendly products and production processes.

Helmholtz Zentrum München – German Research Center for Environmental Health – Control of Regulatory Networks (CoReNe)

The interdisciplinary network known as CoReNe (Control of Regulatory Networks) combines molecular biology and theoretical disciplines. It deals in particular with the role of what are known as non-coding RNAs (ncRNAs) with a special focus on the influence of these RNAs on genetic expression and thereby on the cell's regulatory networks.



Stem cells

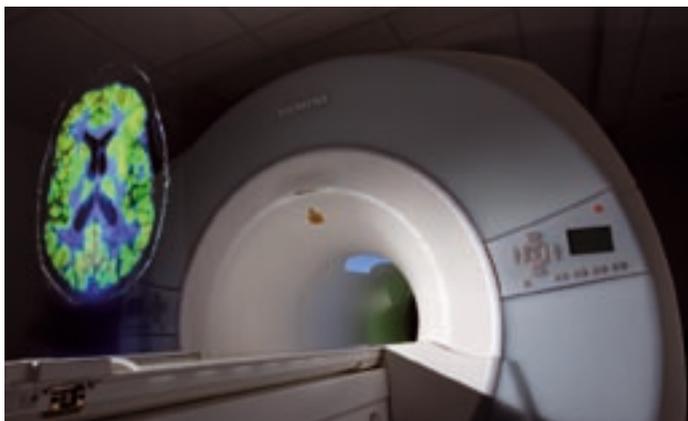
Photo: Helmholtz Zentrum München

THE CENTRE:

The unity of humankind and the environment is the focus of the Helmholtz Zentrum München – German Research Center for Environmental Health. As a result, the work of the centre concentrates on linking ecological and biomedical research, which is a unique concept in the German research landscape. Three central tasks determine the work of the Centre's 1,800 employees in Munich: understanding the basics of human life, comprehending what the requirements for good health are and developing healthcare concepts accordingly. From this understanding, it should be possible to identify health risks for people and threats to their ecosystems at an early stage, decipher mechanisms of disease genesis, estimate limits to the burdens that our environment and human defence mechanisms can withstand and draw up concepts for lasting prevention and cure.

Forschungszentrum Jülich – The Human Brain Model

The Human Brain Model network investigates structure function relationships in the human brain. The brain is analysed as a complex system that extends from nano to macro. Special attention is paid to structural basics and functional mechanisms in interaction because their cooperation determines the functions of the brain. The project is organised in three interlinked work packages. Its aim is to integrate data from all areas and thereby to gain a better understanding of the mechanisms of the healthy and diseased brain.



THE CENTRE:

The Forschungszentrum Jülich conducts cutting-edge interdisciplinary research. The Centre tackles the most pressing issues of our time while at the same time developing key technologies for tomorrow. Research concentrates on healthcare, energy and the environment, and information technology. With about 4,000 employees, Jülich – a member of the Helmholtz Association – is one of the largest research centres in Europe.

A hybrid system consisting of a magnetic resonance tomograph with a field strength of 3 Tesla combined with a positron emission tomograph (3-T-MR-PET)

Photo: Forschungszentrum Jülich

Helmholtz Centre for Infection Research

The Helmholtz Centre for Infection Research (HZI), together with the TU Braunschweig, is setting up the Braunschweig Integrated Centre for Systems Biology (BRICS) where scientists from both institutions will jointly conduct research into infection and immunity and issues of biotechnology. Particular focus is currently being placed on analysing molecular networks in biofilm formation and interaction between regulatory T cells.



Marita Sylla examining an agar plate with bacteria

Photo: Helmholtz Centre for Infection Research

THE CENTRE:

The Helmholtz Centre for Infection Research conducts research aimed at solving the challenges of infection research and making a contribution toward public health by means of new strategies to prevent and treat infectious diseases. To ensure that patients benefit from research findings as fast as possible, the HZI with its 700+ employees operates a joint research centre with the Hannover Medical School and is a co-founder of the Translation Alliance in Lower Saxony.

News from the BMBF

Federal Government increases expenditure on research and innovation

The 2010 federal budget, which was adopted in late March, provides for an increase in the BMBF budget of roughly €660 million to a total of €10.87 billion. This represents an increase of 6.5% over 2009. The Federal Government has thus taken the first step towards achieving the goal defined in the Coalition Agreement to invest an additional €12 billion into education and research during this legislative term. This ensures a sound financial basis for continuing the Higher Education Pact, the Excellence Initiative and the Pact for Research and Innovation. Funding for the life sciences is increasing by roughly 12% to almost half a billion euros.

Third EFI Report: Support for innovation and growth strategy

The Expert Commission on Research and Innovation (EFI) set up by the Federal Government presented its third report on research, innovation and technological performance to Federal Chancellor Angela Merkel and Federal Research Minister Annette Schavan last February. The experts explicitly support the Federal Government's strategy of promoting innovation and growth and emphasize the importance of education and research in future government action. The report concludes that Germany stands out against other countries including the US and major EU countries owing to the increases implemented in spending on research and innovation in recent years.

The Commission recommends a stronger focus on particularly important fields such as climate/energy, health, mobility, communication, and security as well as an improved commercialization of the results of publicly funded research. Referring to the education system, the Commission points to some problems which have resulted from the introduction of Bachelor and Master programmes at German universities as part of the Bologna reform. The Federal Government will join forces with the *Länder* to improve the situation.



Federal Minister Schavan with participants at the 2010 Cluster Conference (Source: BMBF)

Cluster conference of the BMBF – Five new leading-edge clusters launched

450 high-level experts from politics, business and science met at the 2010 Cluster Conference in Berlin. The presentations and discussions focused on policy decisions for research and innovation in Germany and provided fresh impetus for future strong cooperation between science and industry. The five winners of the second round of the Leading-Edge Cluster Competition were presented with their prizes by Federal Research Minister Annette Schavan and Professor Andreas Barner, chair of the selection panel. The new clusters supplement the existing leading-edge clusters that were created in 2008. The third round of the competition will start in late 2010. Companies and science institutions in a region cooperate closely within the clusters to ensure the quick commercialization of innovative research results.

Stronger links between companies and researchers: New Industry-Science Research Alliance starts work

The Industry-Science Research Alliance is a central body advising the Federal Research Minister. Its members are high-level experts from business and science whose task is to develop forward-looking



projects that will help Germany play a leading role in solving global problems. The Alliance is chaired by Prof. Dr. Hans-Jörg Bullinger, President of the *Fraunhofer-Gesellschaft*, and Dr. Arend Oetker, President of the *Stifterverband für die Deutsche Wissenschaft e.V.* The work of the Industry-Science Research Alliance focuses on the central challenges of the future – climate, energy, health, mobility, security, and communication. It is a living example of close cooperation between researchers and companies – a central objective of the Federal Government’s High-Tech Strategy for Germany. One important project which the Alliance will tackle is the development of a sustainable urban infrastructure with the aim of realizing an energy self-sufficient city.

Three new Humboldt Professors selected

The new Humboldt Professors work in the fields of philosophy, quantum optics and chemistry. This international award with its prize money of up to five million euros per prize winner is presented by the Humboldt Foundation and financed by the BMBF. The Humboldt Professorship honours leading international researchers from all disciplines who are working abroad. The prize money enables them to continue their research at a German university under working conditions that can compete internationally, thereby strengthening the profile of research in Germany. Nominations are made by the German universities where the award winners are to establish their working groups.

A further three Humboldt Professors were added in February 2010 to the 15 who have been selected since 2008:

- **The Austrian-born philosopher Hannes Leitgeb from the University of Bristol will establish a new centre for mathematical philosophy at Ludwig-Maximilians-Universität in Munich.**

- **Ulm University nominated the physicist Dietrich Leibfried, who is working in the field of quantum optics and is an expert on ion traps. He will head a newly established quantum technology institute at Ulm University.**
- **The US-born chemist Alec Wodtke will move to Göttingen, where he will become the founding director of a newly established centre for energy conversion.**

German-Brazilian Year of Science 2010

In December 2009, Federal Chancellor Angela Merkel and Brazilian President Luiz Inácio Lula da Silva announced the “German-Brazilian Year of Science, Technology and Innovation 2010/11”. The aim of this Science Year is to promote even closer cooperation between both countries, addressing such issues as global climate change and encouraging growth and innovation. This will provide a lasting impetus for German-Brazilian science cooperation. The Science Year was officially launched in April 2010. It will offer numerous events in Brazil and Germany, which will mainly focus on the exchange of talented young researchers. One example is the Brazilian-German Workshop on Systems Biology that was held in Ouro Preto in the Brazilian state of Minas Gerais in April. The workshop, which was supported by the *Deutsche Forschungsgemeinschaft* (DFG) and the BMBF, enabled 29 researchers from Germany to meet with their colleagues in Brazil and discuss possible approaches for future cooperation projects.

News from the BMBF

Extension of research cooperation with the United States

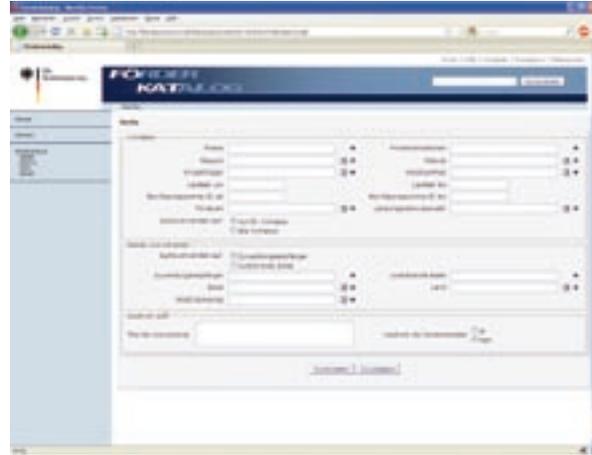
Germany and the USA will cooperate even more closely in the field of research. In February 2010, Federal Research Minister Annette Schavan, the German Ambassador to the US, Klaus Scharioth, and Deputy Secretary of State James B. Steinberg signed an Agreement on Science and Technology Cooperation as a political basis for achieving this goal. This agreement provides a framework for exploiting the potential of transatlantic cooperation even more consistently. It covers a number of research areas and aims particularly to support the establishment of cooperative activities in health research. In the area of regenerative medicine, for instance, the BMBF is already supporting specific cooperation projects with partners in the US.

A “German Center for Research and Innovation” was opened in New York immediately after the signing of the agreement in order to increase the international visibility of German science. The Center will demonstrate the diversity and excellence of research in Germany. Similar centres, initiated by the BMBF and the Federal Foreign Office, have been opened in São Paulo, New Delhi, Moscow and Tokyo. The centres serve as a hub for the international activities of German research organizations and universities and support expanding German businesses in establishing representative offices abroad.

Funding Catalogue of the Federal Government provides an insight into German science

The website www.foerderkatalog.de provides an overview of public funding by the BMBF and other federal government departments.

The Funding Catalogue is a database for the general public that offers a variety of information. It enables searches across more than 110,000 ongoing or concluded projects receiving federal project grants. Users can search for specific topics of funding or for projects conducted at a specific institution.



The Funding Catalogue enables systematic searches for research projects funded by different government departments.

An international approach to studying climate change

The BMBF launched the new framework programme “Research for Sustainable Development” with the aim of supporting Germany’s contribution to climate change mitigation and promoting sustainable developments on a global scale. Activities under the programme will address the global challenges of climate change and water scarcity, the threats to biodiversity, and raw materials and energy supply. Possible solutions will be developed in cooperation with international partners.

In particular, the programme, which is funded with more than €2 billion for the period up to 2015, will support cooperative activities with developing countries in Africa and with emerging economies such as Brazil, Russia, India, China and South Africa. Furthermore, increasing support will be provided for climate mitigation research – €650 million will be invested for that purpose in the expansion of German research vessel capacity over the next six years.



Treading new paths in aging research

Demographic change in Germany will soon be strongly felt in everyday life. Leading a self-determined life is an important topic for many families in Germany. As people live longer, they are more likely to develop diseases such as dementia, metabolic diseases and cancer. Studying the biological causes of aging together with age-related diseases is therefore of special importance to social systems and society at large. As a key technology of the life sciences, systems biology will be a major driver of innovation in the health sector in the coming decade. For the first time, it offers an opportunity to raise our knowledge of the dynamics and interaction of human life processes to an entirely new level. The BMBF programme “Systems Biology for Health in Old Age – GerontoSys” supports research projects which provide a systematic record of the complex interaction of age-related processes in humans. A first round of calls for proposals in 2009 served to combine the relevant expertise of the new branch of systems biology with the established discipline of aging research in Germany. This led to the emergence of one research node and two application-centred cooperation projects:

- **Jena Centre for Systems Biology of Aging – JenAge: “Systems biology of mild stress in healthy aging – a multi-species approach”**, coordinated by Dr. Jürgen Sühnel, Leibniz Institute for Age Research (Fritz Lipmann Institute) Jena
- **“Mitochondrial network of pathways in aging and lifespan control – a systems biology approach (GerontoMitoSys)”**, coordinated by Professor Heinz D. Osiewacz at Goethe Universität Frankfurt
- **“Stromal aging”**, coordinated by Professor Petra Boukamp at DKFZ Heidelberg.



GerontoSys serves to study the prerequisites of healthy aging. (SyB – Fotolia.com)

The follow-up call “GerontoSys 2”, which was issued in March 2010, has supplemented and extended the existing funding opportunities. The aim is not only to establish another one or two research nodes and application-centred cooperation projects, but also to start supporting up to 10 junior research groups in order to lastingly strengthen systems biology research on aging in Germany.

Source: PtJ (Project Management Jülich)

Contact

Information on these and other interesting topics of the High-Tech Strategy for Germany is available at www.hightech-strategie.de

SYSTEMS BIOLOGY OF HUMAN DISEASE

an INTERNATIONAL CONFERENCE

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Registration opens: March 1, 2011
Registration deadline: May 16, 2011
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Poster abstract deadline: May 16, 2011
Additional presentations will be chosen from
submitted poster abstracts

Confirmed Speakers include:

Philippe Bastiaens - Max Planck Institute of
Molecular Physiology, Dortmund

Tim Hucho - Max Planck Institute for Molecular Genetics, Berlin

Arthur Lander - University of California, Irvine

Andre Levchenko - Johns Hopkins University, Baltimore

Susan Lindquist - Massachusetts Institute of Technology

Avi Ma'ayan - Mount Sinai School of Medicine, New York

Marius Ueffing - Helmholtz Zentrum München

Michael White - University of Liverpool

Michael Yaffe - Massachusetts Institute of Technology

Follow-up conferences:

June 2012 - Heidelberg, Germany
June 2013 - Boston, MA

Sponsored by:

Gaining networks of knowledge from image files

Definiens company profile

By Maria Athelougou, Owen Feehan, Ralf Schömeyer, Günter Schmidt and Gerd Binnig

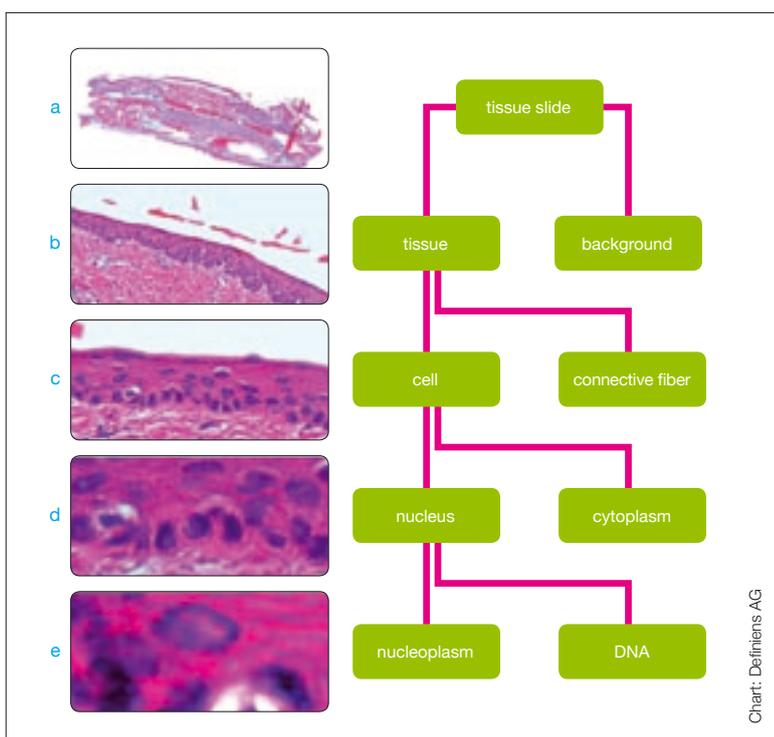
Quantifying structures in biological systems by means of image files provides valuable information about their function and dynamics. New and more powerful imaging processes are generating increasingly enormous quantities of image data. These images are of sub-cellular structures, cells, cell groups, tissue, organs and organisms, but this data is only of any value if information or knowledge can be gained from it. That is why automatic analysis of image data and the extraction of information from it have a key role to play in biology, medicine and drug discovery and development.

The software company Definiens AG has set itself the target of automating and simplifying the analysis of image data. For this purpose Definiens has developed the Cognition Network Tech-

nology, which simulates human cognition processes and incorporates expert knowledge and contextual information into the analysis. Use is made of the image objects and their allocation to a hierarchical image object network (Fig. 1), a semantic network and a process network. This principle of this context and object-based approach is independent of scale and problem. That is why it is eminently suitable for studying biological systems. The Definiens Cognition Network Technology can handle image data in different formats, sizes, dimensions, resolutions and modalities. This approach has already proved its worth in analysing electron, optical and confocal microscopy and radiology (CT, PET/CT, MRI, US) images.

To simplify the development of these solutions there is a semantic computer language – cognition network language (CNL) – based on cognition network technology. It can be used to develop

Fig. 1: The hierarchical content of a medical-biological image



The image of the tissue slide (a) consists of background and tissue (b). The tissue consists of the connective tissue and the cells (c). They in turn consist of cell nuclei and cytoplasm (d). The cell nucleus contains nucleoplasm and DNA (e). Images (a) to (e) are all linked to each other via the level immediately above or below an image. In the hierarchical network all objects carry information about their direct and indirect surroundings. Cognition network technology generates a classified image object network from this overall picture.

solutions based on an analysis of the images interactively as scripts. The developers have a user-friendly interface. Segmentation and classification algorithms, variables and parameters can be defined at the click of a mouse and combined flexibly.

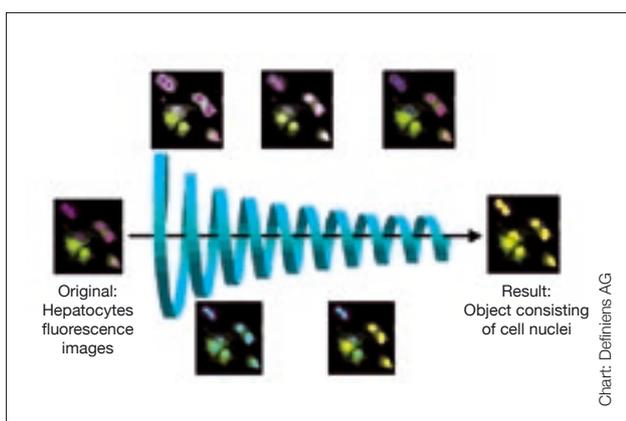
An evolutionary and self-similar process

In contrast to conventional image analysis methods that extract information by means of predefined pixel operations and sophisticated filters, CNL image analysis is an evolutionary and mostly local process. The image segment, which represents the target object, is generated iteratively via several intermediate target objects. This gradual “emergence” of the target object can be illustrated as a spiral. In Fig. 2 the large spiral represents the global analytic process that results from local processes. They in turn are controlled in the image by the local context and local properties arising as a result. As each local process can consist

of not just one single process but may incorporate local sub-processes, the spiral is “self-similar” and corresponds to a fractal hierarchical system. This “evolutionary approach” has proved advantageous mainly in connection with complex image analysis problems. The results consist not only of the properties of individual pixels and pixel regions but also of networked objects and classes and the relationships between them.

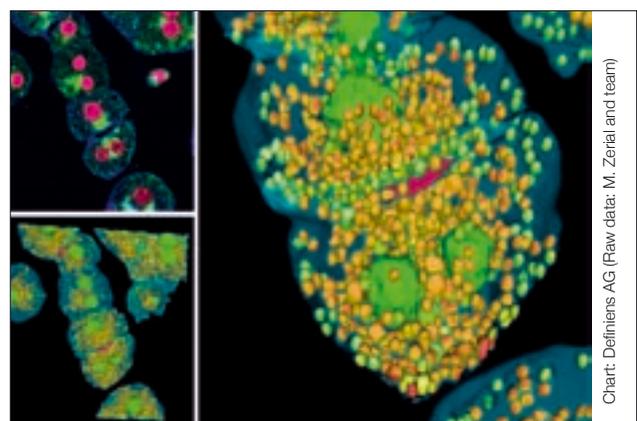
In systems biology knowledge is generated mainly by data analysis, mathematical modelling, simulation and in-vitro and in-vivo experiments. In this process, image data is increasingly establishing itself as the information carrier between experiment, modelling and simulation. Analysing it provides important parameters for both models and experiments. The parameters generated from image data then serve as input for experiments and the accompanying models and simulations.

Fig. 2: Image analysis as an evolutionary and self-similar process



The image of the tissue slide (a) consists of background and tissue (b). The tissue consists of the connective tissue and the cells (c). They in turn consist of cell nuclei and cytoplasm (d). The cell nucleus contains nucleoplasm and DNA (e). Images (a) to (e) are all linked to each other via the level immediately above or below an image. In the hierarchical network all objects carry information about their direct and indirect surroundings. Cognition network technology generates a classified image object network from this overall picture.

Fig. 3: Three-dimensional analysis and illustration of experimental image data by means of CNL



Individual hepatocytes (grey), cell nuclei (green), endosomes (brown, white) and canalicula (red). Original (above left).



The authors (from left to right): Maria Athelougou, Günter Schmidt, Gerd Binnig, Owen Feehan

Mediator between experiment, modelling and simulation

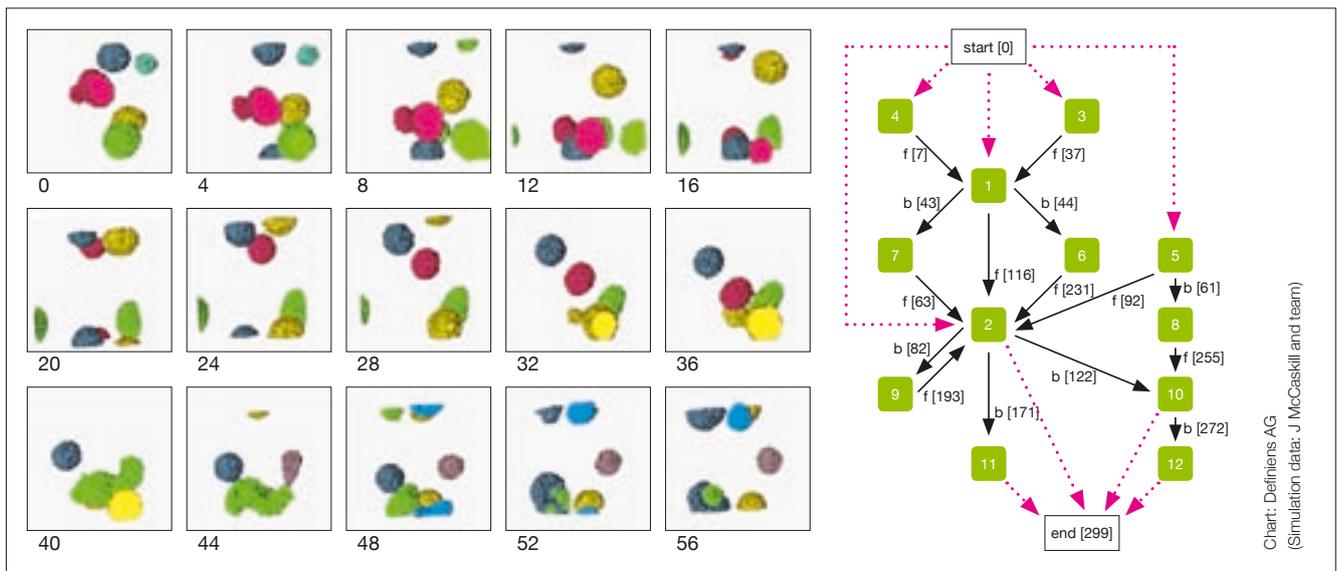
Simulation usually results in alphanumeric data, such as space-time coordinates, representing endosomes. These cell organelles develop during endocytosis in which an area of the cell membrane is pushed into the interior of the cell. A distinction is drawn between early and late endosomes. Early endosomes are found in the cell periphery, late endosomes near the cell nucleus (Fig. 3). Using CNL, data from simulation can be converted into images that are then analysed just like experimental image data (Fig. 4). The aim of this approach is to homogenise the findings of experiments and simulations and make them comparable. Image analysis thus becomes a “catalyst” of interaction between experiment, modelling and simulation. In the HepatoSys/EndoSys research network funded by the German Federal Ministry of Education and Research, this

process is put to exemplary use to analyse endocytotic processes in the hepatocytes (Figs. 3 and 4).

In systems biology images are usually produced using different modalities. If the liver is being examined, for example, tissue images are obtained by means of an optical or confocal microscope, phase contrast images of living cells and ultrasound, CT, MRI, PET/CT images are taken of the entire liver and the entire organism. All of this image data can be processed iteratively by means of CNL. The first step involves analysing certain image objects. These intermediate findings are then used as contextual information for further analysis in another modality.

If the available image data fails to provide suitable contextual information, other data sources such as data from genetic and clinical investigations can be included in the analysis. The concept of

Fig. 4: Analysis of simulation results using CNL



Left: Automatic object segmentation, classification and object tracking of vesicles with the aid of a CNL script. The figures represent snapshots of the simulation. Each object is assigned to a class of its own that is colour-coded here. Right: An automatically generated graph shows what happens in vesicular development. b [time] denotes development processes (budding), f [time] vesicular fusion and start [0] and end [299] the beginning and end of simulation.

multimodal image analysis thereby gains an entirely new quality. Valuable networked knowledge is generated that advances our understanding of biological systems and, for example, makes new diagnostic procedures or customised drugs possible.

Company profile in brief:

Definiens AG is a software company founded in Munich in 1994 by Nobel laureate Prof. Gerd Binnig. He and his team developed Definiens Cognition Network Technology®, an object and knowledge-based and context-driven technology that is used most successfully for image data analysis in biology, medicine and the geosciences.

The work presented was undertaken as part of the systems biology project HepatoSys (EndoSys Network) funded by the German Federal Ministry of Education and Research: <http://endosys.mpi-cbg.de/index.html>

Research partners: Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Dept. Marino Zerial; Mannheim University Hospital, Molecular Research in Gastro-Enterology, Dept. Steven Dooley; Ruhr University Bochum, Biomolecular Information Processing (BioMIP), Dept. John McCaskill

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do host cells limit the influenza vaccine production?

The role of the cellular innate immune response on process yields

By Timo Frensing and Udo Reichl

Image: Margaret Jaszowska – Fotolia.com

The most efficient protection against influenza viruses is provided by vaccination. Since the current vaccine production capacities are limited, cell culture-based processes are currently being established to compensate for increasing demands for vaccines. Although parts of the vaccine production in bioreactors are well-characterised with respect to cell propagation, metabolism, and cell culture media, little is known about the impact of the host cell's pathogen defence system on the yield of vaccine production.

Influenza viruses are enveloped RNA viruses and belong to the family of *Orthomyxoviridae*. In particular, the influenza A and B viruses represent a high burden for human health. Each year, influenza viruses cause 2 up to 5 million infections in Germany, and about 8.000 to 11.000 of all infected people die (estimated by the German Robert Koch Institute).

The pathogenicity or virulence of influenza viruses is primarily caused by their huge genetic variability. Due to high mutation rates, the viruses constantly change and modify their surface proteins, which enable them to escape from recognition by the host immune response - a process called antigenic drift. Further-

Fig. 1: Cellular signalling cascades induced through influenza A virus infection that are relevant in cellular influenza defence (scheme according to literature reviews).

Viral components: green; genes: yellow; phosphorylation events chosen for analysis: blue; interferon induced proteins: pink (CellDesigner™ Ver. 3.5, The Systems Biology Institute, Tokyo, Japan).

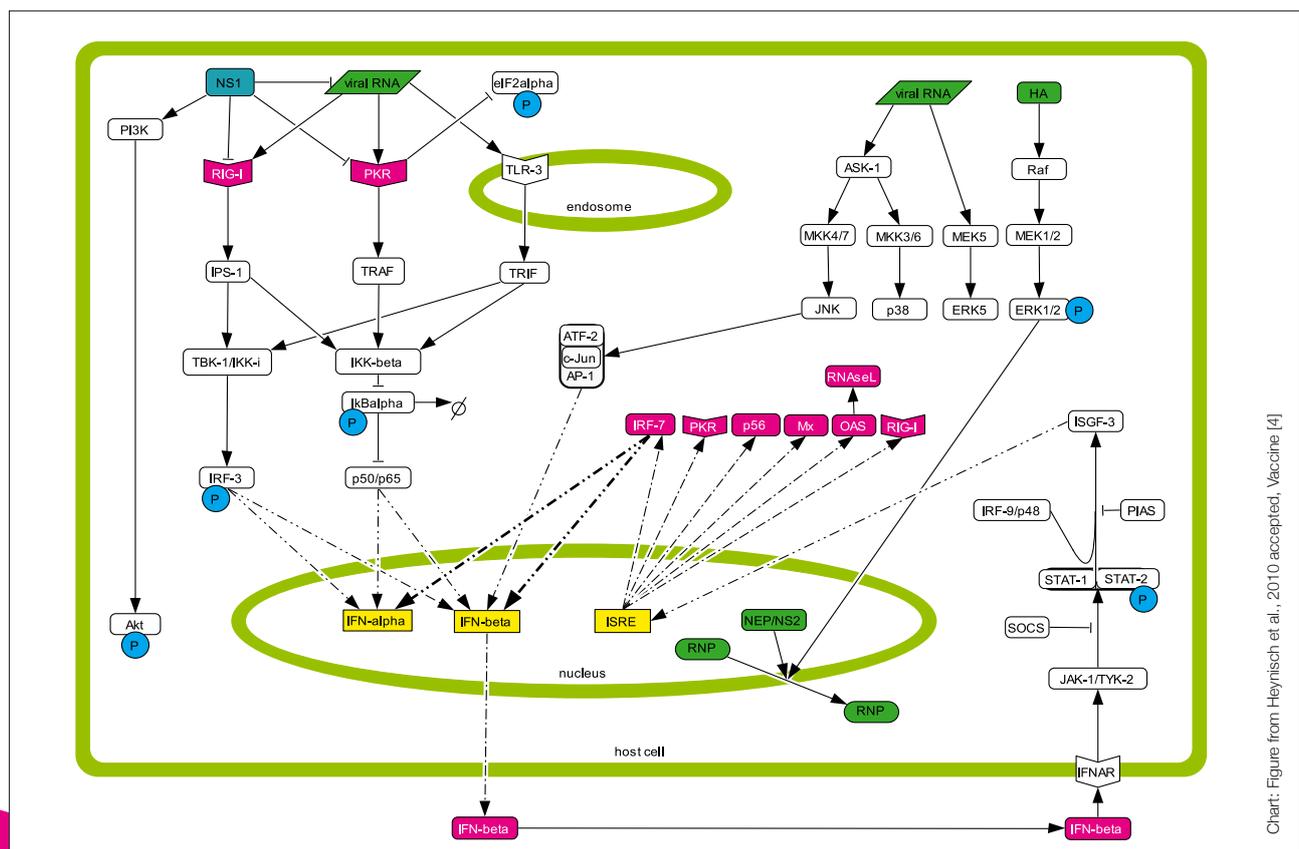


Chart: Figure from Heynisch et al., 2010 accepted, Vaccine [4]

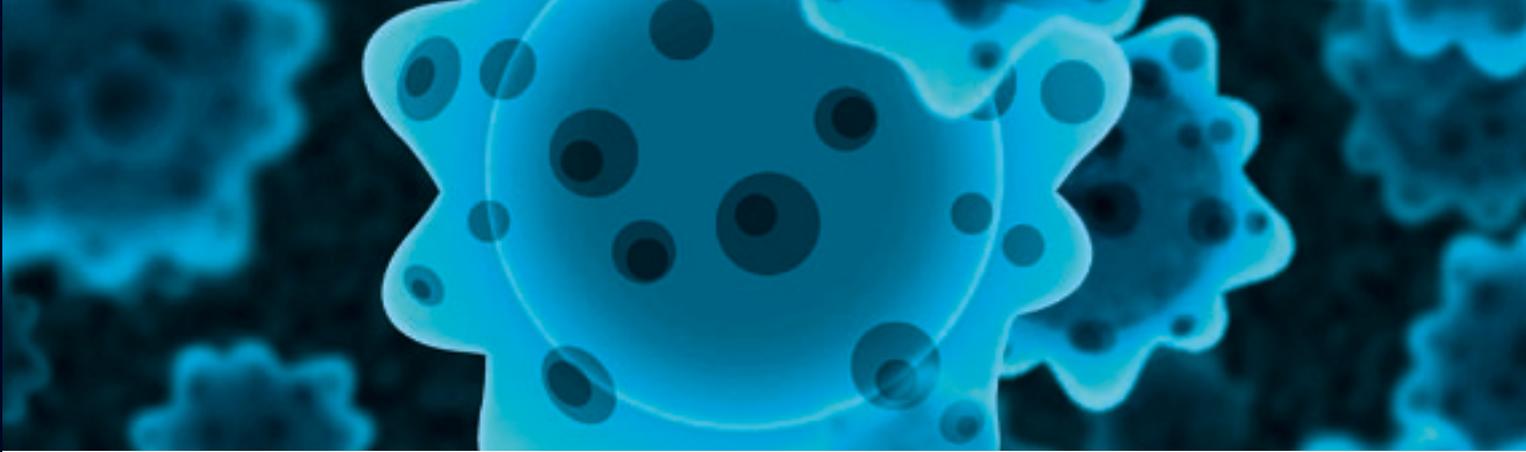


Image: Margaret Jaszowska – Fotolia.com

more, the segmented RNA genome of influenza viruses allows for the recombination of genomes when a host is infected by two different virus strains. This so-called antigenic shift has the potential to generate reassortant viruses with completely new immunogenic properties and/or even higher mortality.

Another level of complexity is added to this by the fact that besides humans, influenza viruses can also infect other animals like pigs or horses. Interestingly, the main reservoir for influenza viruses is represented by aquatic birds. For example, the 2009 pandemic influenza strain was shown to be a combination of genome segments originating from swine, avian, and human (Novel Swine-Origin Influenza A H1N1 Virus Investigation Team, 2009).

Especially many younger people showed little immunity against this emerging so called H1N1 2009 variant. Hence, an above-average number of children and young adults was affected by this type of flu as compared to elderly people. Fortunately, it turned out that the mortality rate of this virus strain was not as high as initially expected. Nevertheless, this pandemic impressively demonstrated the threat of a fast worldwide virus-spread by human-to-human transmission. This becomes particularly true, since other influenza viruses had shown a much higher fatality rate in the past, like e.g. the pandemic H1N1 virus from 1918 (Spanish flu) or the H5N1 avian influenza virus.

Currently, two classes of antiviral drugs are approved for the treatment of severe influenza infections. On the one hand these are the M2 ion channel inhibitors Amantadin and Rematidine, and on the other hand these are the neuraminidase inhibitors Zanamivir and Oseltamivir. Unfortunately, the frequent use of these drugs already facilitated the development of resistant influenza virus variants.

Hence, the most effective way to prevent severe disease caused by influenza virus infections is vaccination. The phenomena of antigenic drift and antigenic shift, however, circumvent a longer lasting immunity. Thus, annual vaccination is recommended using a trivalent vaccine composition, which contains the three prevalent circulating influenza virus strains.

The majority of these influenza vaccines is still produced in embryonated hen eggs. This production process is well-established, but is certainly not flexible or scalable enough to satisfy the increasing demands at a pandemic situation. Besides, in case of a human pandemic caused by a high-virulent avian influenza strain, the vaccine production may collapse due to the loss of laying hens. Evaluating the current situation, the existing production capacities are insufficient to produce a trusted supply of vaccines for a worldwide coverage within one year. These shortfalls became apparent in the H1N1 2009 pandemic when the

Fig. 2: Microscopic picture of influenza virus-infected MDCK cells

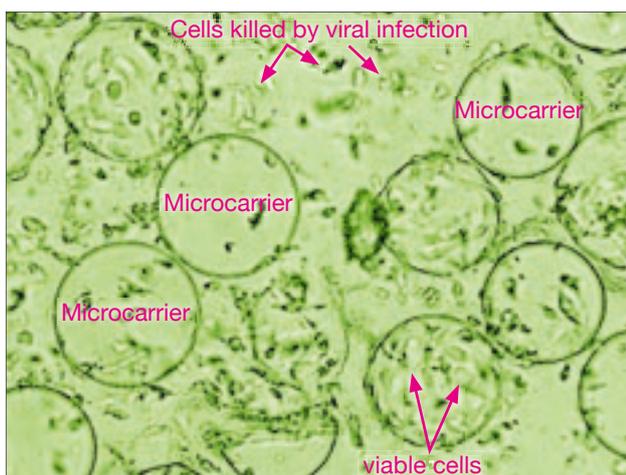
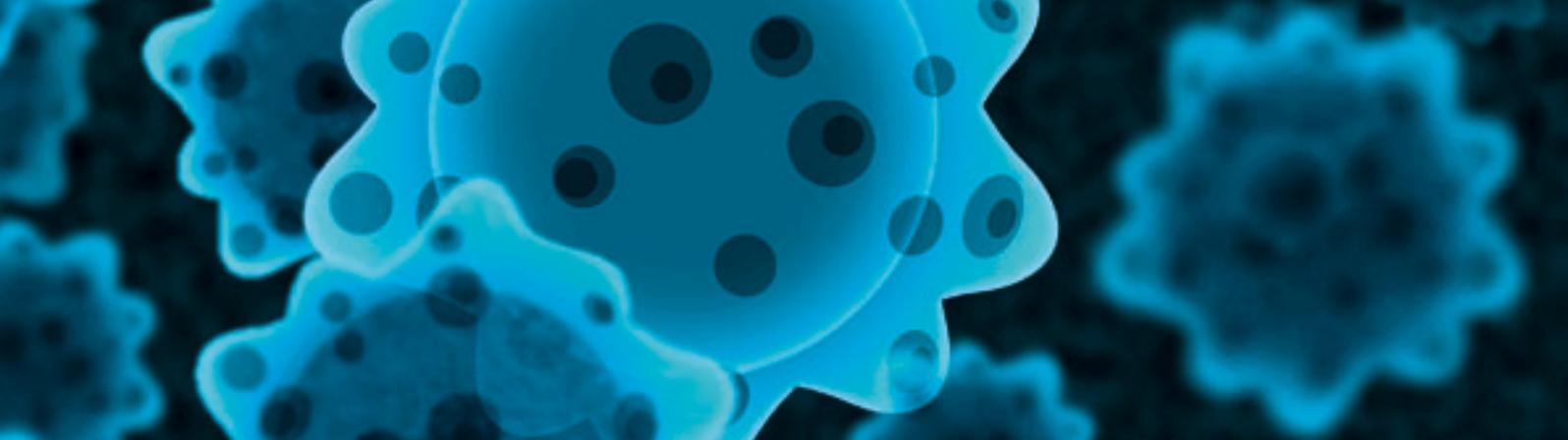


Chart: Udo Reichl, University of Magdeburg

MDCK cells were propagated in bioreactors on microcarriers (small dextran beads with a diameter of about 200 μm). Viable cells are attached to the microcarriers in a single layer; infected cells detach and eventually deteriorate. Before cells die they release thousands of new virions (not visible at this magnification), which can be used for the influenza vaccine production.



pandemic vaccine had to be produced in a short time frame and in parallel to the trivalent seasonal vaccine.

To overcome these limitations of vaccine production, vaccine manufacturers are currently establishing cell culture-based production processes. Continuous mammalian cell lines are propagated in bioreactors and thus, in a scalable and controlled environment. At an adequate density, cells become infected with influenza viruses and after 2 to 3 days the supernatant can be further processed to purified inactivated influenza vaccines.

Understanding virus-host cell interactions is crucial for the development of new anti-viral drugs as well as for the optimisation of cell culture-based vaccine production. Therefore, the bioprocess engineering group at the Max Planck Institute for Dynamics of Complex Technical Systems in Magdeburg (Germany) not only investigates cultivation strategies to optimise the vaccine production, but also analyses virus-host cell interactions in bioreactors. Among others experimental approaches, we tested different cell lines for their ability to produce influenza vaccines. One major focus of our research is directed towards Madin Darby Canine Kidney (MDCK) cells, a cell line that is well-known to be highly permissive for infection with different influenza virus strains [1].

Our group was able to show that the infection and replication dynamics in MDCK cells are highly dependent on the specific influenza virus strain used for infection. In addition, we showed that selected virus strains differentially induced programmed cell death, called apoptosis, in their host cells. All of these factors significantly determine the yield of virus production [2]. In order to gain a better understanding of the virus replication in a qualitative and quantitative manner, these data were used to develop mathematical models of virus infection, replication, and propagation in cell cultures.

The experimental results showed that influenza virus strains not only differ in the extent of apoptosis induction, but also induce different responses on protein expression level within the host cells. A protein that was found to be up-regulated in virus-infected MDCK cells is the antiviral myxovirus resistance protein 1 (Mx1) [3]. This result has brought up the question, how these strain-specific host responses are induced and whether the enhanced expression of antiviral proteins influences yields in the vaccine production?

To tackle this question, we analysed the intracellular signal transduction pathways of MDCK cells, in particular, those pathways that are part of the innate immune response and that are related to virus-sensing and subsequent activation of the cellular pathogen defence system (Figure 1). A key role in the innate immune response to pathogens is played by interferon signalling that is known to activate the expression of more than 300 genes for cellular pathogen defence system. The results showed that the used influenza virus strains induced these signalling pathways to a significantly different extent [4]. In addition, some of the analysed pathways are related to apoptosis induction, explaining the observed differences mentioned above.

In order to analyse the influence of these defence mechanisms on the yield of virus production in a bioreactor, we manipulated the cellular signal transduction system. First, we activated the pathogen defence by interferon-stimulation of MDCK cells. When the stimulated cells were infected, we observed a delay in virus replication. However, no differences were found with respect to the final virus yields. In an opposite approach, we inhibited the induction of the pathogen defence of MDCK cells. Again, virus yields were almost equal. This low influence of the cellular pathogen defence was quite unexpected and raised the question whether MDCK cells possess an antiviral capacity at all.

Mx proteins, identified in our protein expression studies, also play a crucial role in the influenza defence of humans and mice. Hence, the antiviral activity of Mx proteins of MDCK cells was

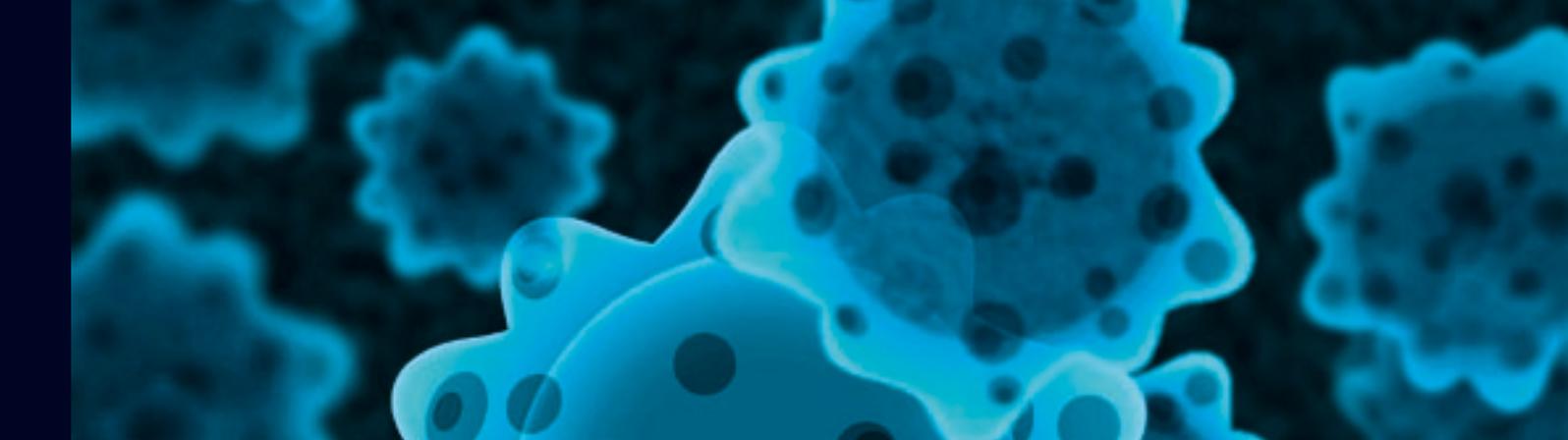


Image: Margaret Jaszowska – Fotolia.com

analysed in collaboration with the group of Prof. Dr. Georg Kochs (Department of Virology, University Freiburg). Interestingly, and in contrast to the human MxA or the mouse Mx1, the Mx proteins of MDCK cells showed no antiviral activity. This lack of inhibitory potential of Mx proteins against influenza virus replication makes MDCK cells an ideal system for high yield vaccine production [5].

The research project in brief:

Project name: FORSYS-project WPB 9 „Dynamics of Influenza A Virus Replication in Epithelial Cells“, funded by the German „Bundesministerium für Bildung und Forschung“ (BMBF)

Max-Planck-Institute for Dynamics of Complex Technical Systems, Magdeburg, Chair of Bioprocess Engineering, Otto von Guericke University Magdeburg
www.mpi-magdeburg.mpg.de

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exploring the immune system

Systems biology research networks in quest for new therapy options

By Thomas Höfer, Jonathan Lindquist, Burkhard Schraven and Wolfgang Schamel

The immune system is one of the most complicated organs of the human body, providing reliable protection from the continuous assault by bacteria, viruses and parasites. However, the immune system's blessing can become a curse when immune cells become hyperactive or even begin to attack the body's own structures. This can lead to allergies, asthma and debilitating autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. It is becoming clear that these diseases result from a complex interplay of molecular and cellular mechanisms that is beginning to be investigated with the methods of systems biology. In this article, the outcome of interdisciplinary collaborative research projects leading to recent findings by the authors are presented.

Therapeutic interventions in the immune system are sought to treat a variety of diseases

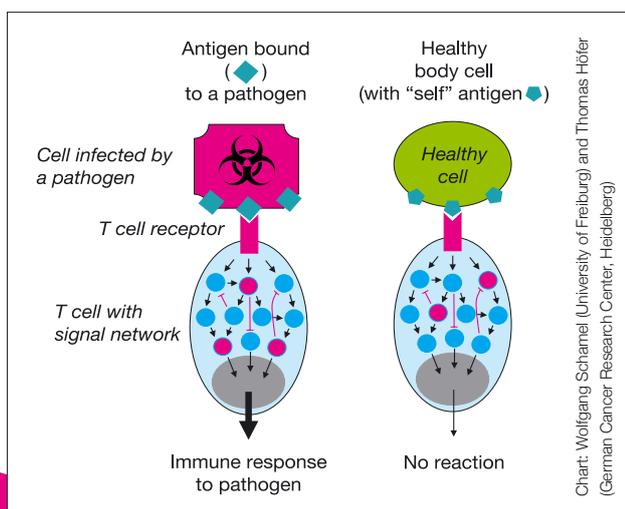
An ideal therapy for autoimmune diseases would be to eliminate the dysfunctional immune response to the body's own structures without affecting the vital defence mechanisms against invading pathogens. However, current therapies aimed at suppressing

immune responses are far from achieving this ideal. They are usually not very selective and are often accompanied by strong side-effects.

More effective and specific methods for suppressing immune responses are also required for patients who undergo organ transplant surgery. To prevent the body from rejecting a transplanted 'foreign' organ, patients need to be treated with immunosuppressive drugs on a long-term basis.

However, there are also diseases which could be treated by deliberately activating immune responses. This strategy is particularly promising for cancer therapy. The immune system often identifies cancer cells, like pathogens, as structures that need to be combated because they differ from normal body cells and express different proteins on their surface. Nonetheless, the majority of tumours do not trigger strong immune response. We still do not understand very well the reasons for the lacking immunity against tumours. Some tumours seem to actively inhibit immune responses aimed at them. There are thus hopes for new approaches that treat cancer by surmounting these mechanisms and making tumours 'visible' to the immune system again.

Fig. 1: T cells must be able to distinguish between "foreign" and "self"



T cells can distinguish very accurately between antigens derived from pathogens and substances produced naturally in the body ("self-antigens"). The decision whether a T cell is activated, proliferates and fights pathogens or remains inactive is determined by a complex molecular network within the cell.



Image: Sebastian Kaulitzki – Fotolia.com

‘foreign’ and ‘self’ has been investigated particularly well for T cells. The molecular selectivity observed is quite astonishing: A T cell that responds to a certain antigen can ignore a very slightly changed molecule (e.g. with only a 20% weaker binding affinity for the T cell receptor). We do not know of any other cell type in the human body that can discriminate so accurately between different molecules. We hope that a deeper understanding of the underlying mechanisms will provide new molecular targets for immunotherapy.

Regulatory networks mediate decisions on the activation, division, migration and survival of T cells

In recent decades immunologists have worked intensively to identify the protein molecules that mediate the activation of T cells by antigens. Today we have an extensive inventory of the molecules involved and their states of activity. This inventory is being expanded even further by new systems biology methods such as mass spectroscopy. However, the central question which mechanisms enable T cells to distinguish so reliably between antigens and the body’s own molecules has still not been fully resolved (Fig. 1).

This question is difficult, if not impossible, to address with the traditional methods of molecular immunology, focusing on one molecule at a time, because many different molecules interact with each other in complex networks. To obtain a precise picture of the molecular network that governs T cell activation, immunologists, systems biologists and mathematicians headed by one of us (B.S.) in Magdeburg have compiled extensive knowledge from the scientific literature and their own research, evaluated it critically, and stored it systematically. The resulting “topological” model describes the signalling pathways like a map that lead from the T cell receptor and two co-receptors to the activation of genes and to the regulation of cell proliferation.

The first version of the model comprises of 96 different molecules linked by 129 connections (biochemical reactions) (Fig. 2). Importantly, this model has predicted previously unknown signalling routes (pathways in the map) that were subsequently

confirmed by experiments. In addition to the antigen stimulus, T cells integrate many other signals that they receive from other cells and that help to determine when cells divide, to which parts of the body they migrate, and for how long they survive. We are now continuously extending and updating the molecular map to include these additional signalling pathways. Our analyses of the routes taken by activating and inhibitory signals in this extensive regulatory network give rise to novel problems that we are tackling in close cooperation with mathematicians.

T cell receptors cooperate

To understand the mechanisms of cellular decisions, different levels of molecular organisation, from the individual molecule to large networks with thousands of molecules, need to be investigated. Our research into the mechanisms of how the antigen stimulus is passed through the cell membrane adds another level of molecular complexity to the signalling network map. One of us (W.S.) has recently demonstrated that T cell receptors cooperate in clusters. When cooperating T cell receptors (but not individual receptors) are activated by the binding of antigen from the outside of the cell, a change in the three-dimensional structure of the receptor molecules inside the cell is triggered. These observations can explain how information about the existence of pathogens passes through the cell membrane and stimulates the complex signalling networks in the cell. We (T.H. and W.S.) have recently teamed up to develop a mathematical model of this process and measure crucial molecular parameters needed by the model. The model shows how cooperation of T cell receptors enables T cells to distinguish reliably between ‘foreign’ and ‘self’. The computational analysis of the model has led here to new, and sometimes counterintuitive, predictions that were subsequently validated experimentally.

Dynamics matter

Dissecting the spatio-temporal dynamics of signalling networks stimulated by the T cell receptor and other receptors on the surface of the T cell has turned out to be crucial for understanding their biological function. In cooperation with Andreas Radbruch and Max Löhning at the German Rheumatism Research Centre in

Berlin one of us (T.H.) is studying the molecular machinery that controls the division and differentiation of T cells.

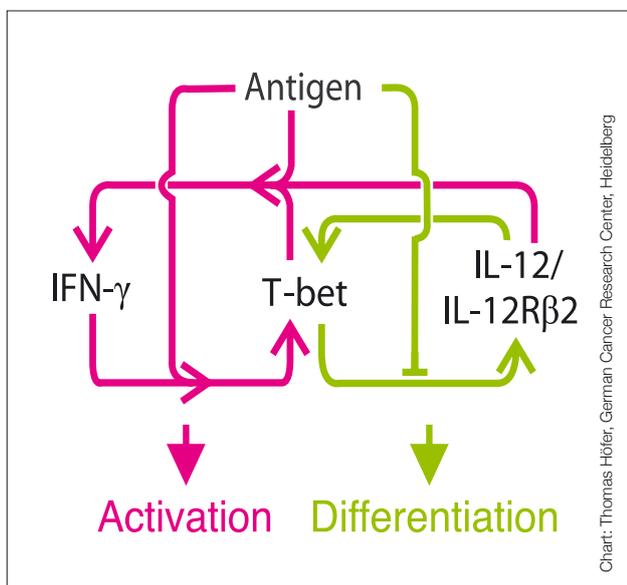
By iterating between time-resolved experiments and computer simulations of mathematical models, we found major gaps in our understanding of the process by which ‘naïve’ T cells differentiate, upon antigen contact, into effector and memory cells that can efficiently combat viruses or tumours. This work initiated new experiment that led us to discover hitherto unknown molecular interactions. As a result, we have now a quantitative, predictive model of the gene module that governs this differentiation pathway of T cells (Fig. 3). The model shows how the level of a master regulator, the T-bet protein, is controlled by a web of positive and negative interactions. As such master regulators also govern other differentiation pathways of T cells (and, indeed, many other cell types of the human body), the iteration between kinetic experiments and mathematical modelling will be more widely applicable to help decipher gene regulation.

Computer simulation can help us reveal emergent phenomena in regulatory networks. These dynamic processes cannot be understood from the properties of individual molecules alone but require the study of their dynamic interaction. We have dem-

onstrated such an emergent phenomenon – a ‘bistable’ switch – that determines whether an antigen-stimulated cell divides or not. It is not triggered directly by the T cell receptor but by an intermediate signal produced by the T cells themselves. This signal – the diffusible messenger molecule interleukin-2 – stimulates cell division in a positive feedback loop through activating the expression of the interleukin-2 receptor. This feedback leads to an all-or-nothing reaction that only permits sufficiently strong antigen signals to trigger cell division. These results help us to understand better how yet another class of T cell, the regulatory T cell, functions to prevent autoimmune responses.

Although we are only beginning to understand the regulation of T cell activation at the systems level, these examples already show how new and medically relevant insights can be gained by combining biological experiment and mathematical theory. We are currently collaborating closely with many colleagues in research networks to develop models of T cell activation and differentiation at multiple scales of spatio-temporal organization, including signal transduction, gene regulation and cell-to-cell communication. These models integrate data from the molecular level to cellular function and lead to new and testable predictions on how the immune system functions (or malfunctions) in health and disease.

Fig. 3: Feedback controls activation of T cells



The core network of differentiation of T cells into memory cells capable of efficiently combating viruses and tumour cells consists of two interlinked feedback loops (cf. Schulz et al 2009 Immunity 30, 673). The magenta-coloured loop shows the activation of cells in the presence of antigens, while the green-coloured feedback loop is crucial for differentiation. This network integrates the antigen signal and the stimuli that are triggered by the messengers interferon- γ (IFN- γ) and interleukin-12 (IL-12). We arrived at this network by combining time-resolved experiments and mathematical modelling in iterative loops.

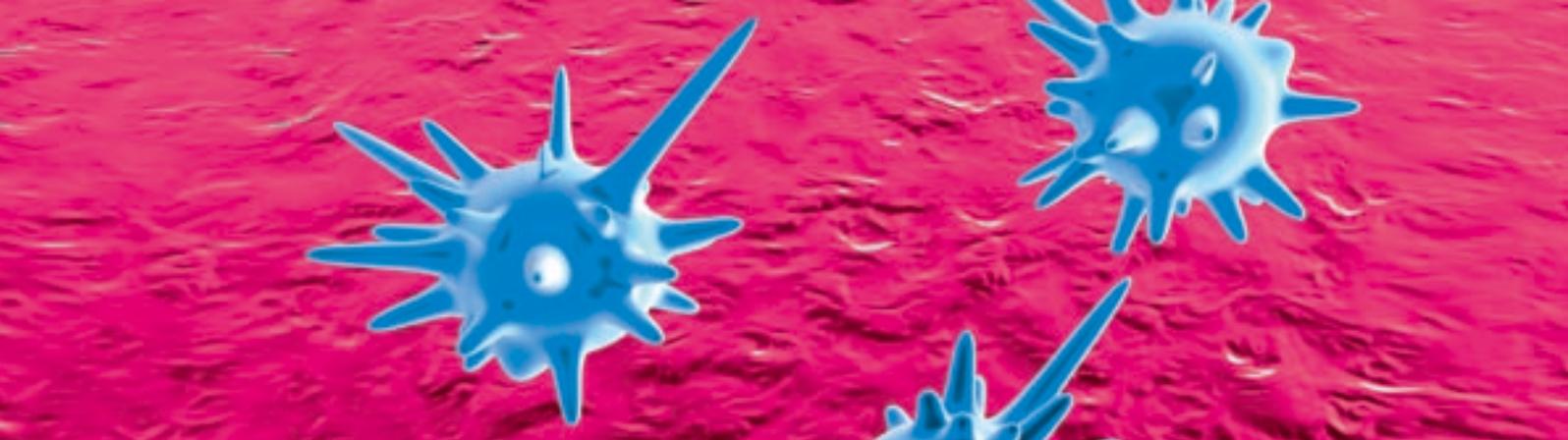


Image: Spectral-Design – Fotolia.com

The research project in brief:

Project name: SYBILLA – Systems Biology of T Cell Activation in Health and Disease

A consortium of 18 European work groups funded by the Seventh EU Framework Research Programme. The authors of this article are members of the SYBILLA consortium, which is coordinated by Prof. Dr. Wolfgang Schamel.

www.sybilla-t-cell.de/

Project name: Magdeburg Center for Systems Biology (MaCS), funded as a systems biology unit (FORSYS Centre) by the German Federal Ministry of Education and Research (BMBF). The MaCS project on the systems biology of T cells is headed by Prof. Dr. Burkhard Schraven.

www.mpi-magdeburg.mpg.de/MaCS

Project name: Transcriptional Programming of T cells

A FORSYS partner group at the German Rheumatism Research Centre, Berlin (coordinator: Dr. Ria Baumgraß with Prof. Dr. Andreas Radbruch and Prof. Dr. Max Löhning), the German Cancer Research Center and BioQuant Center, Heidelberg (Prof. Dr. Thomas Höfer) and other organisations in close cooperation with MaCS and the Heidelberg-based FORSYS Centre “ViroQuant”.

Project name: Helmholtz Alliance on Systems Biology

A Helmholtz Association project involving six Helmholtz Centres and more than ten other partners. The German Cancer Research Center in Heidelberg coordinates the Alliance and heads the network SBCancer – Systems Biology of Signalling in Cancer, which incorporates Prof. Thomas Höfer’s work group.

www.helmholtz.de/systembiologie

Associated partners:

Centro de Biología Molecular Severo Ochoa Madrid: Prof. Balbino Alarcon; Helmholtz Centre for Infection Research, Braunschweig: Dr. Hansjörg Hauser; German Rheumatism Research Centre and Charité Universitätsmedizin Berlin: Prof. Dr. Max Löhning, Prof. Dr. Andreas Radbruch; Harvard Medical School, Boston: Prof. Dr. Anjana Rao

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the weight of the biomarker

Bruker Daltonik company profile

By Detlev Suckau and Ralf Ketterlinus

The Bremen-based company Bruker Daltonik GmbH is a pioneer in developing mass spectrometric analysis techniques for biomedicine. In 2010, the company, with its 500 employees, can look back on a 30-year tradition of developing mass spectrometers for biochemical, pharmaceutical and clinical applications and is currently developing new and more powerful clinical diagnostic procedures (Ref. 1).

A mass spectrometer is a high-tech “balance” that can weigh individual molecules. The molecular weight, as calculated from the measurements of molecular properties, enables molecules to be detected in tissue that can indicate the presence of diseases such as diabetes, bacterial infections and cancer. In a histological tissue section, e.g., proteins can be made visible that identify certain tumour cells as biomarkers. This involves determining the spatial distribution of the molecular species in question across the tissue surface – the mass spectrum (Fig. 1).

Advancing to the molecular dimension

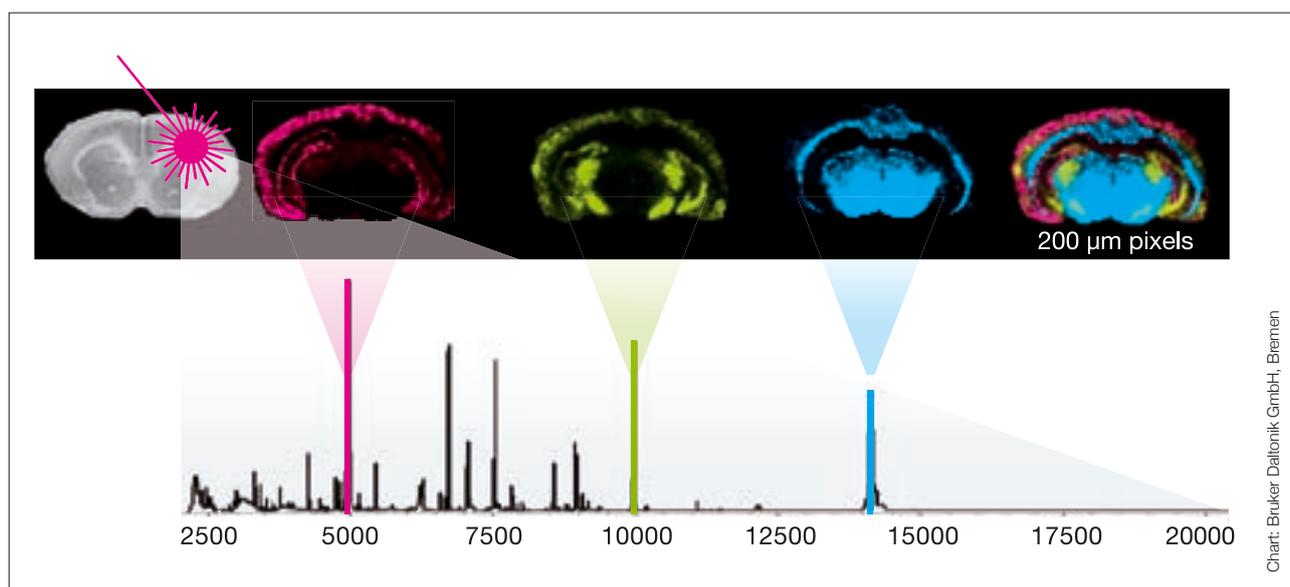
The mass spectra of all pixels together provide for the distribution of the molecule in question in the tissue section. This technology is known as MALDI imaging. It may still be in its early days, but it has enormous potential for systems biology, proteomics and medicine (Ref. 2).

MALDI stands for “Matrix-Assisted Laser Desorption/Ionisation”. It can be used to vaporise molecules that normally do not vaporise but instead only become “scorched” (rather like meat on a grill) when heat is applied to them. This procedure can be performed in the vacuum of the mass spectrometer – an ideal method for investigating biomolecules such as proteins or lipids.

These analyte molecules are embedded in a chemical matrix. Desorption occurs when the matrix is vaporised by means of laser pulse irradiation. The analyte molecules are dragged into the gas phase and protonated by the matrix, i.e., they become charged. In the mass spectrometer the molecules are then accelerated by an electric field until they hit a detector.

Fig. 1: The principle of MALDI imaging

For each pixel of a tissue section a mass spectrum is obtained by laser pulse irradiation (below). The intensity distribution of several proteins (green, red and blue peaks) is assigned to the tissue image. Superimposing these false colour images (right) shows the spatial distribution of different proteins in the tissue sample. A cancer marker can be specifically detected and localised in this way.



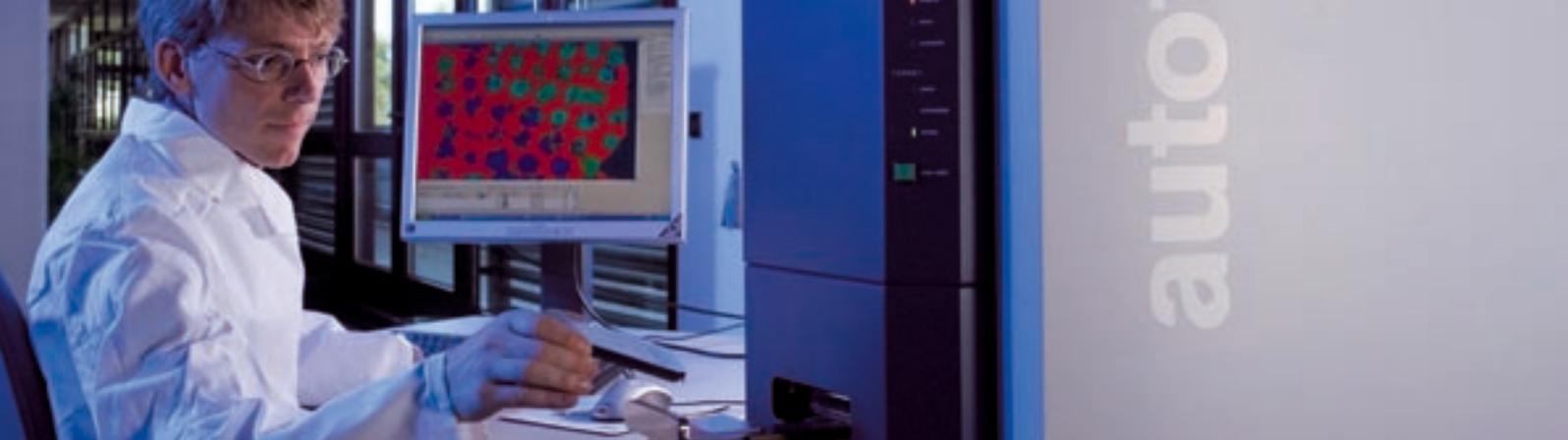


Fig. 2: MALDI mass spectrometer: Modern MALDI mass spectrometers are developed by Bruker Daltonik GmbH integrated with software and in a workflow. Molecular histology is a new application; it is used to determine the distribution of proteins in clinical tissue samples. (Photo: Bruker Daltonik GmbH, Bremen)

Lighter molecules reach the detector faster than heavier ones. The flight times of the molecular ions range from nanoseconds to microseconds! This measuring process is known as time-of-flight mass spectrometry or time-of-flight (TOF). MALDI-TOF was discovered in Germany in the late 1980s and was awarded the Nobel Prize in chemistry in 2002.

An instrument for medical diagnostics

MALDI imaging adds a molecular dimension to histology and enables the distribution of different kinds of molecules to be made visible with almost cellular resolution in tissue samples. Using this method, new HER2 breast cancer markers were discovered from tissue samples (Ref. 2). As a world-leading technology provider, Bruker Daltonik can offer a complete biomarker detection and identification workflow for tissue samples – from sample preparation and measurement to statistical evaluation of patient data.

The technology is being further developed within the BMBF-funded SYSTEC project to increase the speed of imaging and achieve an image resolution that is capable of depicting individual cells. A further objective is to develop statistical algorithms so that it is possible to identify and classify cancer automatically using MALDI imaging. The project is a continuation of the groundbreaking research into MALDI imaging that Bruker Daltonik has been pursuing since 2005. MALDI-TOF mass spectrometry is expected to gain increasingly in importance in histopathology and clinical microbiology.

Company profile in brief:

A medium-sized enterprise that has been involved for 30 years in the development of mass spectrometers and especially in the development of mass spectrometric analysis techniques for biochemical, pharmaceutical and clinical applications. It is actively participating in a network project as part of the BMBF funding focal point New Methods in Systems Biology (SysTec) with the aim of developing new, more powerful and less expensive clinical diagnostic procedures.

www.bdal.de

Associated partners: Helmholtz Centre Munich; Institute of Human Genetics: Marius Ueffing; Institute of Pathology: Axel Walch, Heinz Höfler; Institute of Biological and Medical Imaging (IBMI): Vasilis Ntziachristos; Institute of Biomathematics and Biometrics: Rupert Lasser; Definiens AG: Günter Schmidt; Bruker Daltonik Bremen: Detlev Suckau

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live cell RNA interference screens – an effective way from gene to function

The ViroQuant-CellNetworks RNAi screening facility at the BioQuant Center

By Holger Erfle

RNA interference (RNAi) allows the expression of individual genes to be actively suppressed, thereby leading to effective knock-downs of gene function. This method combined with novel automated transfection technologies and automated microscopy enable geneticists to take a huge step forward in deciphering gene function in living cells. Here we introduce the activities of the RNAi Screening Facility at BioQuant, the Center for Systems Biology at Heidelberg University.

At present RNAi is the method of choice to study loss-of-function phenotypes by suppressing the function of virtually any desired protein-coding gene. As in traditional genetics, the phenotype, or externally ascertainable features of human cells, can be determined analysing their morphology (structure and form). Cultured cells enable a uniquely detailed phenotype analysis after gene-knock-down by RNAi using fluorescent pathogens, reporter proteins or immunofluorescence microscopy.

Massive parallelisation

In order to use massive parallelisation in the analysis, a novel solid-phase transfection technology on thin glass slides (cell arrays) [1] and recently introduced, in multi-well plates [2] was established and optimised at the BioQuant Center. The principle of the cell arrays is that different siRNAs being printed onto a glass slide along with a transfection solution in a spatially defined array. These arrays are dried and then coated with adherent mammalian cells. The cells then absorb the different siRNAs at the distinct spots. The result of this is that the expression of the proteins in question is suppressed. Using the high-throughput process the gene-phenotype relationship of many genes can be investigated in parallel. In cell arrays or multi-well plates, gene specific RNAi of all protein-coding genes of the human genome is performed by means of short, double-stranded RNA molecules (21 nucleotide long, small interfering, siRNAs). A genome-wide siRNA library was acquired as part of the BMBF funded FORSYS-ViroQuant project that makes it possible to suppress the expression of 20,203 protein coding genes.

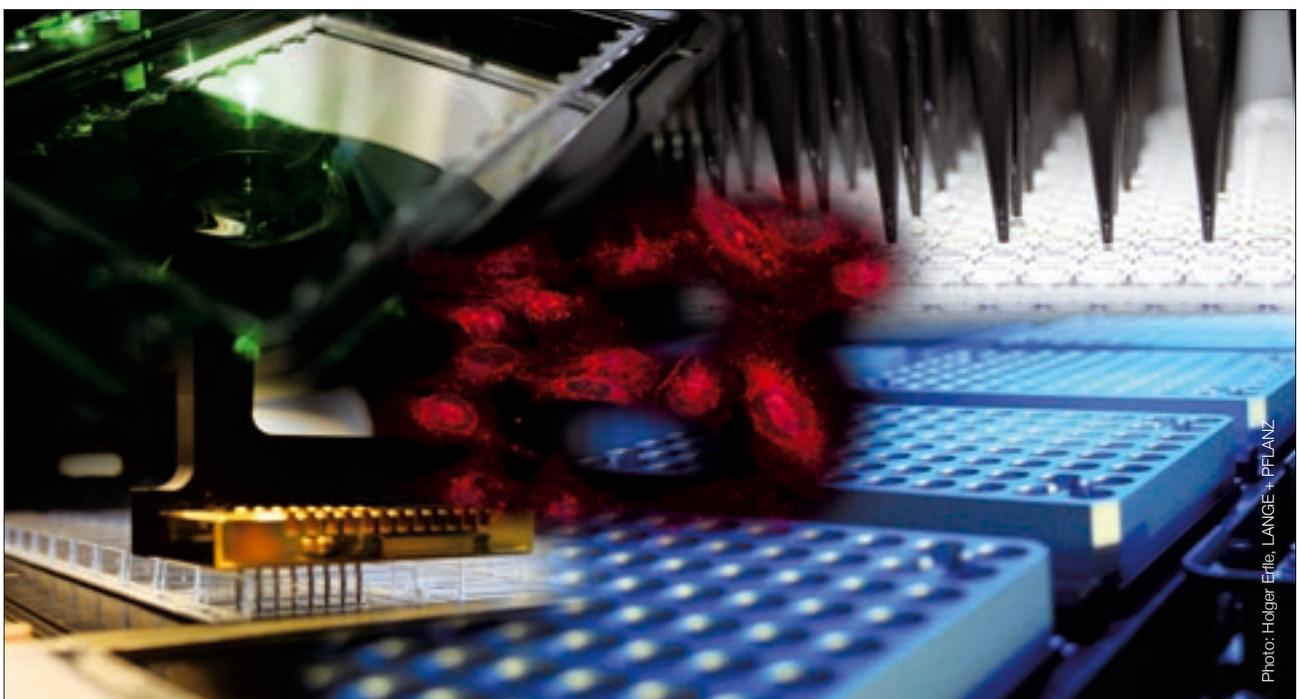


Photo: Holger Erfle, LANGE + PELANZ



Dr. Holger Erfle heads the RNAi-Screening Facility at the BioQuant Center in Heidelberg.

Cutting-edge technology for research

Automated microscopes can target spots on the arrays or holes in the multi-well plates to obtain images of their cell morphology after the use of RNAi. Robotics and microscopes were acquired as part of the ViroQuant project with support from the CellNetworks excellence cluster. The research group provides three wide-field microscopes and two confocal microscopes for producing automated images. These microscopes can be used to measure live cells and are connected to the local server system. The large data quantities are transferred via gigabit lines (fibre-optic systems are currently being developed) to local server systems for automatic analysis and archiving [3]. As part of FORSYS-ViroQuant and in cooperation with the research groups of Hans-Georg Kräusslich and Ralf Bartenschlager at Heidelberg University, human host proteins that play a part in the entry and replication of the viral pathogens HIV (human immunodeficiency virus), HCV (hepatitis C virus) and DV (dengue virus) were identified.

On the way to low-cost RNAi Screening

The number of spots per cell array has been increased in the RNAi screening facility in pilot tests from 384 to 9,216 [4]. The clear objective is to place an entire genome on a cell array and to reduce the cost significantly to only €1,000 per screen, which would bring the method within reach of a larger number of laboratories. High sample densities would also make combined screens and the use of rare patient cell lines possible. The research group is now coordinating two research projects. The German Federal Ministry of Education and Research (BMBF) funds the exploration of RNAi of non-coding genes and the Baden-Württemberg State Foundation funds deciphering the regulatory networks of miRNAs in living cells. Both projects aim to link high-throughput microscopy and high-resolution microscopy. Image processing, statistics and bioinformatics are going to be integrated as part of those projects in order to set up fully automated processes and obtain information efficiently.

The research project in brief:

The ViroQuant-CellNetworks RNAi Screening Facility was set up in 2007 as part of the FORSYS-ViroQuant project and supported by the Excellence Cluster CellNetworks at the BioQuant Center at

Heidelberg University. www.bioquant.uni-heidelberg.de

Associated partners: German Cancer Research Center, Division of Theoretical Bioinformatics, and Heidelberg University, IPMB and BioQuant; Roland Eils; Heidelberg University Hospital, Department of Infectiology and Virology; Hans-Georg Kräusslich; Heidelberg University Hospital, Department of Molecular Virology; Ralf Bartenschlager

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pain sensitisation rerouted

A young project with surprising results

By Tim Hucho

The research network “Modelling of Peripheral Pain Switches” focuses on the engine driving ongoing pain. Funded by the German Federal Ministry of Education and Research (BMBF) the consortium investigates for the first time intracellular signalling modules, so called “nociceptive modules” and their interplay. The project got off with a surprising start. We discovered that the cells own mechanisms can be used to convert pain-intensifying signals into sensitisation-eliminating signals. This opens up an opportunity to reprogramme harmful signalling cascades with the aid of the body’s own signalling networks – a major step along the path towards a mechanism-based pain therapy.

Everyone is familiar with pain. Already thinking about it is unpleasant. What can cause pain? With which of our five senses do we register pain? Why does pain sometimes never stop? Why does a painkiller not work? And why is it that ordinary, everyday stimuli sometimes hurt? Many of these questions we still cannot answer satisfyingly. One reason for this is our limited understanding of the cellular mechanisms underlying pain.

Pain is still a problem of the highest order. An estimated 20% of the population experiences at least one long-lasting period of pain at some stage of their lives. For around three out of every four patients, today’s drugs such as non-steroidal anti-inflammatory painkillers (aspirin) or opiates (morphine) but also many recently developed ones do not provide a satisfying level of relief. In addition, the use of current drugs have often limiting side-effects such as nausea, drowsiness, numbness, increased risk of heart attack as well as often the risk of addiction. Thus, the use of today’s painkillers is seriously restricted. Better understanding of the molecular mechanisms of pain sensitisation and the translation into new therapeutical approaches is urgently needed.

Unpleasant, but essential for survival

What is pain good for anyway? Stimuli that trigger pain, e.g. a heavy object landing on ones thumb, are usually registered by specialised sensory nerves in the peripheral nervous system – so called nociceptors. Nociceptors only respond to very strong stimuli such as great pressure or great heat. Weak everyday stimuli do not activate these nerves. Most nociceptive neurons are not insulated by a myelin sheath. This results in the surprisingly slow transmission speed toward the brain experienced dur-

“Turning down” pain



Photo: asdigital.de – Fotolia.com



Pain is a warning signal that helps to minimise damage to health and prompts us to go to the dentist, for example.
Photo: Klaus Eppel – Fotolia.com

ing the surprisingly slow retraction reflex of a hand placed on a hot plate. Once the activation of the nociceptors finally reaches the brain, an acute sense of pain is triggered. This is of vital importance not only to acutely minimise the tissue damage. Even more importantly, any experience of pain helps to steer clear of similar stimuli and situations in future. Indeed, the inability to feel acute pain can be life-threatening. Recently, the scientific journal *Nature* described the case of a boy who, as a result of a genetic defect, was insensitive to pain. The boy regularly demonstrated how he cut through his arms with knives. He died at the age of 13 when he jumped from the roof of a house, unaware of the adverse consequences [1].

When light touch hurts

But acute pain is only half the story. The other half is of great clinical relevance. Nociceptive neurons can be sensitised [2]. Thereby, the activation threshold drops while the strength of the reaction rises. As a consequence everyday stimuli sometimes even as arbitrary as a mere contact with cloths or an ordinary hot bath can trigger sensations of pain. This sensitisation, also known as allodynia or hyperalgesia, can last for longtime – sometimes days, weeks, and even months or years. For the patients this can be debilitating and effect all aspects of life through secondary effects such as insomnia, restricted mobility up to even disability to work.

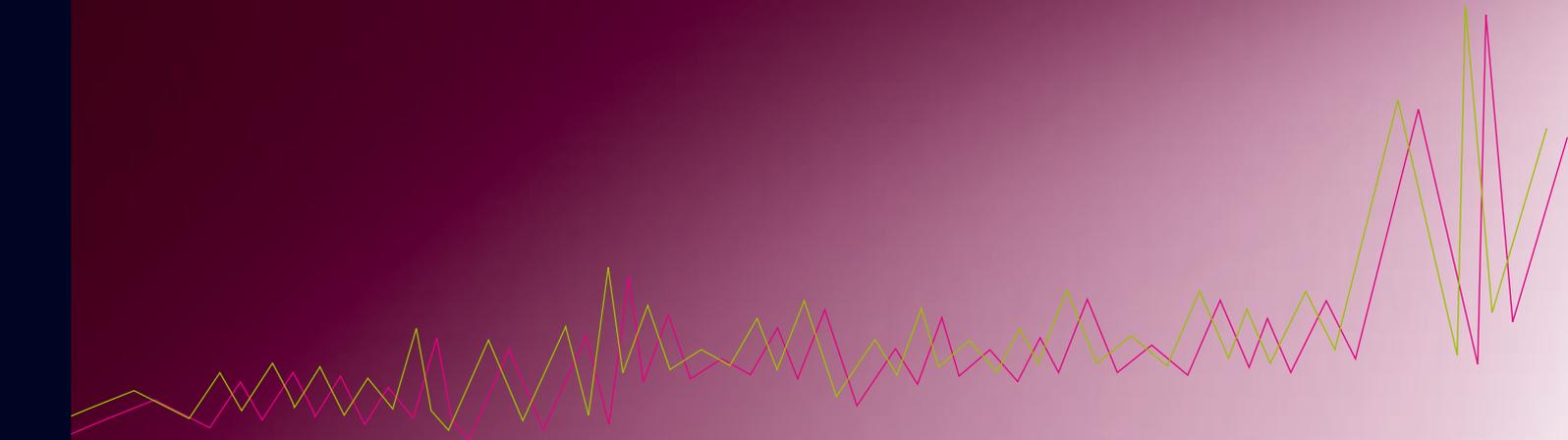
Sensitisation can be caused, for example, by tissue injury in an accident or during operation. Substances secreted during inflammation such as prostaglandin E2, histamine and serotonin as well as growth factors and tissue acidification sensitise nociceptive neurons via many different receptors. Many transient cellular mechanisms such as the phosphorylation of ion channels are initiated. Which signalling pathways in the nociceptors keep the sensitisation motor running is still mostly enigmatic.

Nociceptive Modules

Unlike many pain research projects today, the BMBF-funded “Modelling of Peripheral Pain Switches (Mops)” research network does not focus only on ion channels. Instead, it seeks to disentangle the signalling network behind sensitisation. The basic idea is that similar pain phenotypes result from similar signal events in the nociceptive neurons. As there is a plethora of signalling routes into the cell, each of which results in sensitisations, the existence of jointly used intracellular signalling mechanisms – so called nociceptive modules [2] - was postulated. The young research network took the task to further develop this new concept of nociceptive signalling modules, to characterise novel modules, their interplay in molecular and bioinformatic terms, and finally to put this knowledge to clinical use.

Astonishing first results

Until now, little more than two components per sensitising signalling pathway have been known. Thus the task to define nociceptive modules is currently still rather ambitious. But the analysis of one signalling pathway in which the protein kinase C epsilon (PKCε) plays a central part allowed a first exciting breakthrough [3]. As was shown, substances as different as adrenalin and oestrogen were found to activate signalling cascades that converge on PKCε. Thereby indeed the term “module” was corroborated. The data also revealed a further surprise. We found for the first time that a sensitising signalling pathway splits up into a sensitising and a desensitising module. It was possible to decouple the desensitising module from the sensitising signals and address them individually. This enabled the prevention of subsequent sensitisation attempts. Fascinatingly, it also had the effect of eliminating an already established sensitisation.



Cellular signalling reprograms “sensitising” into “desensitising” pain signals

The desensitising module is of general significance. We have not just described a new module that counteracts an already known cellular event by shutting down the input route for example by internalization of the signalling initiating receptor. But instead, we found that a cell’s signalling past can convert intracellular a sensitising into a desensitising signal! Therefore, unlike systems previously described, the cell is not only able to switch signalling pathways on and off. In our example the signalling pathway stays “on” but is rerouted in such a way that its effect is not to sensitise but to eliminate sensitisation. Thus, while the extracellular situation does not change, e.g. the pain inducing tissue damage and/or inflammation is still prevalent, the reaction of the nociceptive neurons is reprogrammed.

The effect and mechanism of this signal reversal have been replicated in the cellular system as well as in animals and, in promising preliminary experiments, even in humans. Delineating further the molecular, cellular, systems biological, and clinical implications will be exciting. This first finding of the only recently founded research network could prove to be a milestone on the road to a novel treatment approach in mechanism-based pain therapy [4] [5].

The research project in brief:

Project name: Modelling of Peripheral Pain Switches (MopS)
A network project with three-year funding from the BMBF’s Medical Systems Biology – MedSys project which also includes consortiums from industry and academia that deal mainly with cancers, immunology/infection, pain, metabolic diseases, biofilms or chronic wounds.
www.molgen.mpg.de

Associated partners: Ralf Baron (University of Kiel, Neurological Clinic – Neurological Pain Research and Therapy Section); Tim Hucho (Coordinator, Max Planck Institute of Molecular Genetics, Berlin); Monika Heiner (Technical University Cottbus, Institute of Informatics); Friedrich Herberg (University of Kassel, Institute of Biology, Biochemistry Department); Wolfgang Marwan (University of Magdeburg, Institute of Biology); Peter Reeh (University of Erlangen, Institute of Physiology and Experimental Pathophysiology); Johannes Schuchhardt (MicroDiscovery GmbH, Berlin); Christoph Stein (Coordinator, Charité Berlin, Anaesthesiology and Operational Intensive Medicine Clinic); Harald Seitz (Max Planck Institute of Molecular Genetics, Berlin)

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- [4] www.neuro.med.tu-muenchen.de/dfns/
- [5] www.pain-signaling.org

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quote

“To understand biology at the system level, we must examine the structure and dynamics of cellular and organismal function, rather than the characteristics of isolated parts of a cell or organism.”

Hiroaki Kitano, 2002

Science (2002) Vol. 295. No. 5560, S. 1662 - 1664

on the way to a virtual liver

Research network embarks on new phase

By Johannes Bausch

The liver is a fascinating organ. Day by day, it handles over 10,000 different metabolites. It detoxifies the body and has the unique ability to regenerate itself after injuries. Due to its complex metabolic pathways and structures, understanding of the liver presents science with a challenge. Six years ago, HepatoSys, Germany's first systems biology initiative, took up this challenge at a cellular level, and it is now being replaced by the Virtual Liver Network at an organ level.

HepatoSys was launched by the German Federal Ministry of Education and Research in 2004. What took shape was a Germany-wide competence network characterised by common research efforts in standardised cell material and by the intensive exchange of data and experience. The HepatoSys teams have easy access to all research results made by other groups in the network. This is made possible through central data management and a sophisticated intranet. Following the creation of the infrastructure, HepatoSys has been concentrating more and more since 2007 on matters relating to content. Four sub-projects on detoxification, endocytosis, regeneration, and iron metabolism formed the bio-

logical focus. Two other projects concentrated on mathematical and cell biology methods. The aim was to combine experimental results in computer models.

From the cell to the entire organ

HepatoSys has entered a new phase in April 2010: the *Virtual Liver Network*. This network aims to take the next step from the cellular level to a higher scale. Observation will no longer be limited to individual cells but instead will extend to cell structures and liver tissue. In the future, the researchers also want to answer questions relating to the entire organ. The goal is to work out the connections and interactions between the different levels and ultimately create a model that will map core liver processes such as detoxification, signal processing, and cell replication.

The researchers are investigating the state of both, the healthy organ as well as typical diseases. Examples they have chosen are inflammation, fatty liver, detoxification, and liver regeneration. The *Virtual Liver Network* hopes that this will lead to medical benefits. It aims to understand the causes of diseases and to contribute toward the development of customised treatment methods.

Picture of the hepatic vessels from CT scan images enhanced using contrast medium

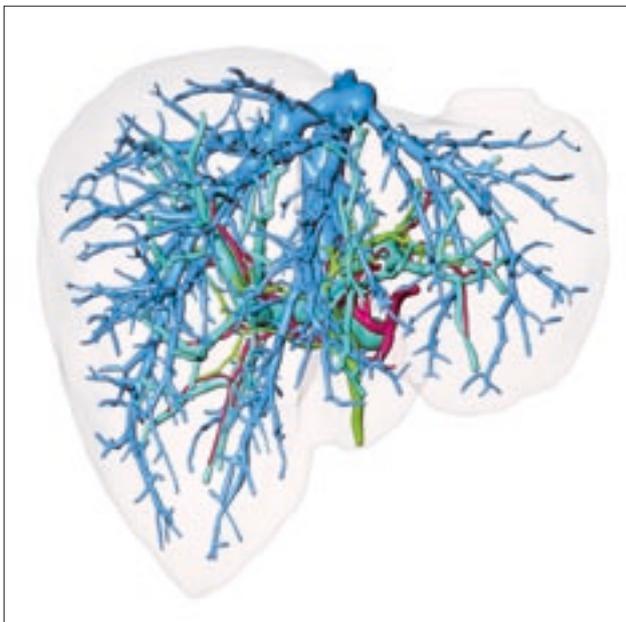
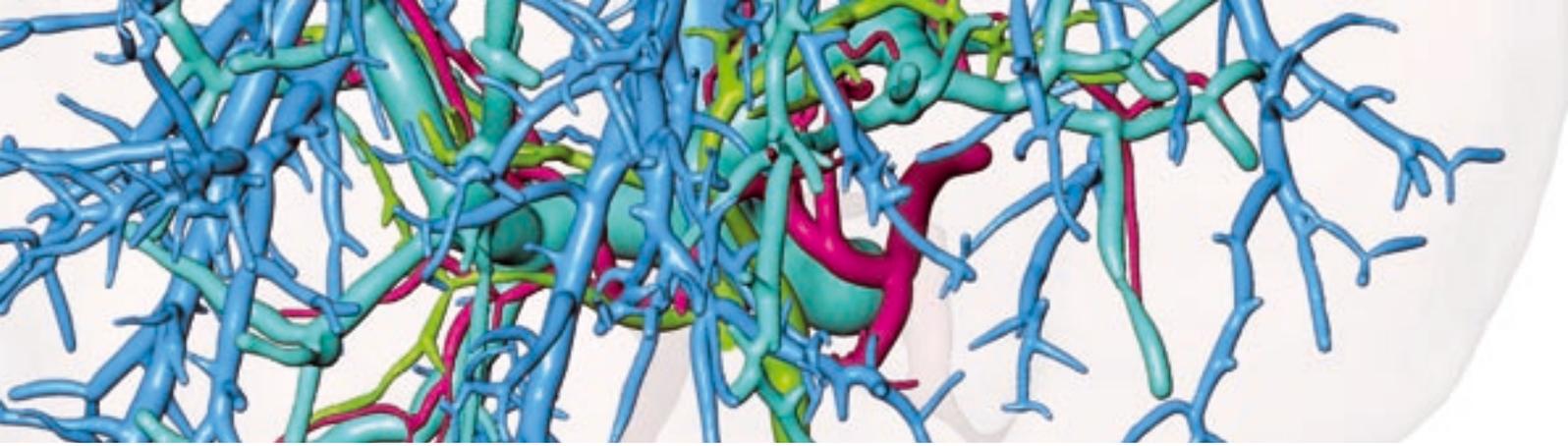


Photo: Fraunhofer MEVIS, Bremen

The liver contains the bile ducts (green) and three vascular systems: the liver arteries (red), the liver veins (blue), and the portal vein (pale blue). These vessels permeate each other and take up the liver's entire volume.



Findings for medicine

The network consists of sub-projects, all of which combine laboratory experiments with modelling. At the same time, data management is developed further and technical standards are established for image processing and analysis of metabolic and protein data. This is because all research findings ultimately end up in a mathematical model – the virtual liver that is the research network’s common objective. The network also aims to build a bridge towards clinical research. New findings on detoxification make their way into medical studies, and the results of these in turn have an effect on basic research.

In the *Virtual Liver Network* almost 70 research groups benefit from over €40 million in funding. The research network’s size and interdisciplinary composition make coordination a real challenge and require a clear structure and strict programme management. An independent programme director supported by the heads of sub-projects and medical experts in liver diseases is expected to handle this task. An advisory council of internationally renowned scientists has been denominated to assist him or her.

Virtual Liver is the first project in the world to integrate findings about an organ from the molecular and cellular level to the organ level. It can serve as a model for future research efforts and enable other organs to be understood.

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Johannes Bausch

Virtual Liver Network

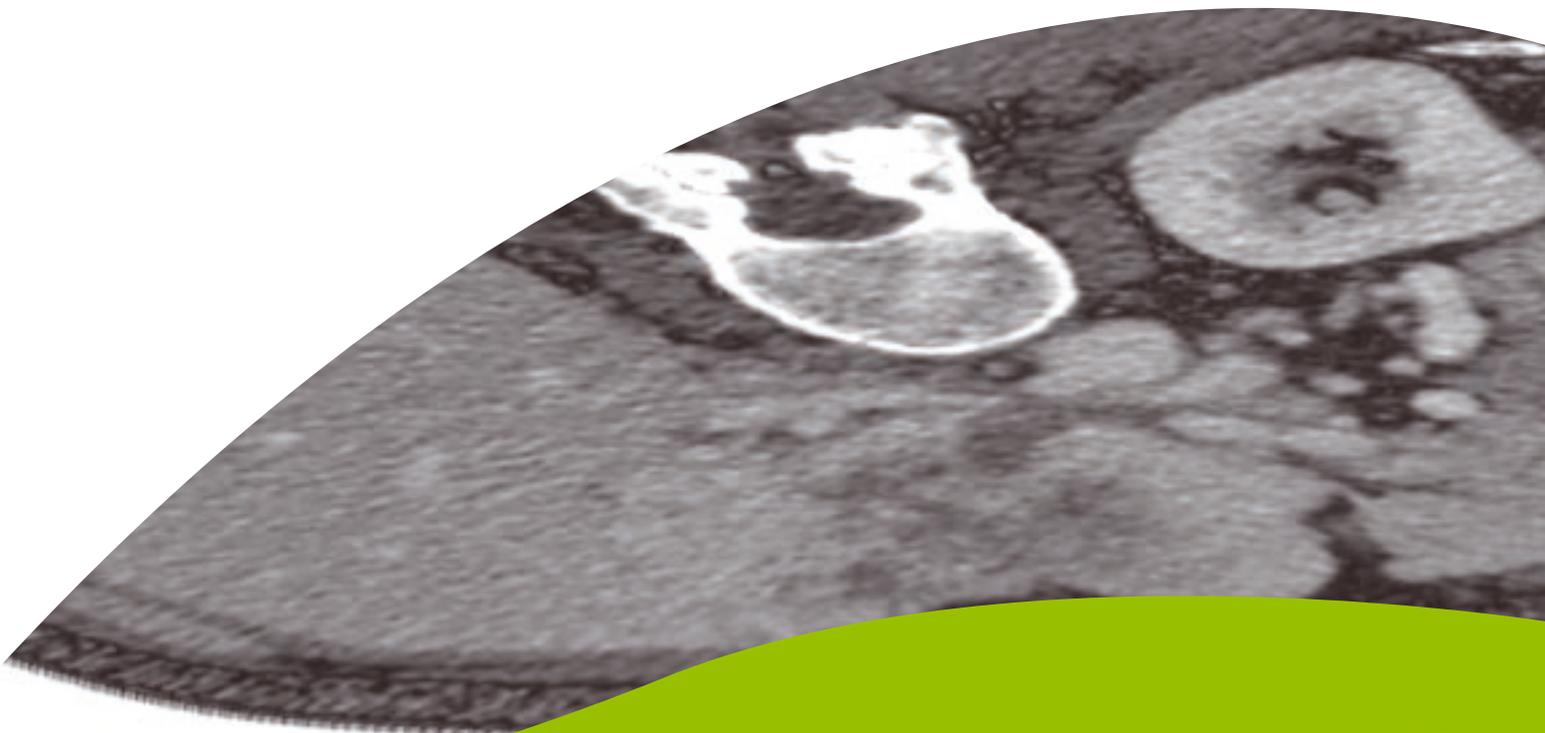
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controlling the development of stem cells

Regulatory networks in the embryo

By Wolfgang Driever and Daria Onichtchouk

Embryonic stem cells have inestimable potential for regenerative medicine because they can create the tissues of all organs. But how can the development of these cells be regulated so as to prevent uncontrolled growth and tumours from occurring? That is only possible if we gain a better understanding of how cell differentiation is regulated in the embryo. A combined approach involving embryology, bioinformatics and systems biology modelling has provided new insights into the first development decisions by embryonic stem cells. In the process, it has been shown that the regulatory mechanisms of fish and mammals have an astonishing degree of similarity.

Stem cells could open up new prospects of a cure for patients with degenerative diseases. Stem cells with the potential to create many different kinds of tissue – pluripotent cells, as they are known – have been met with particular interest. They include embryonic stem cells (ES cells), which in the past could only be extracted from embryonic tissue. In recent years scientists have

succeeded in prompting body cells to convert into pluripotent cells, giving rise to new biomedical expectations.

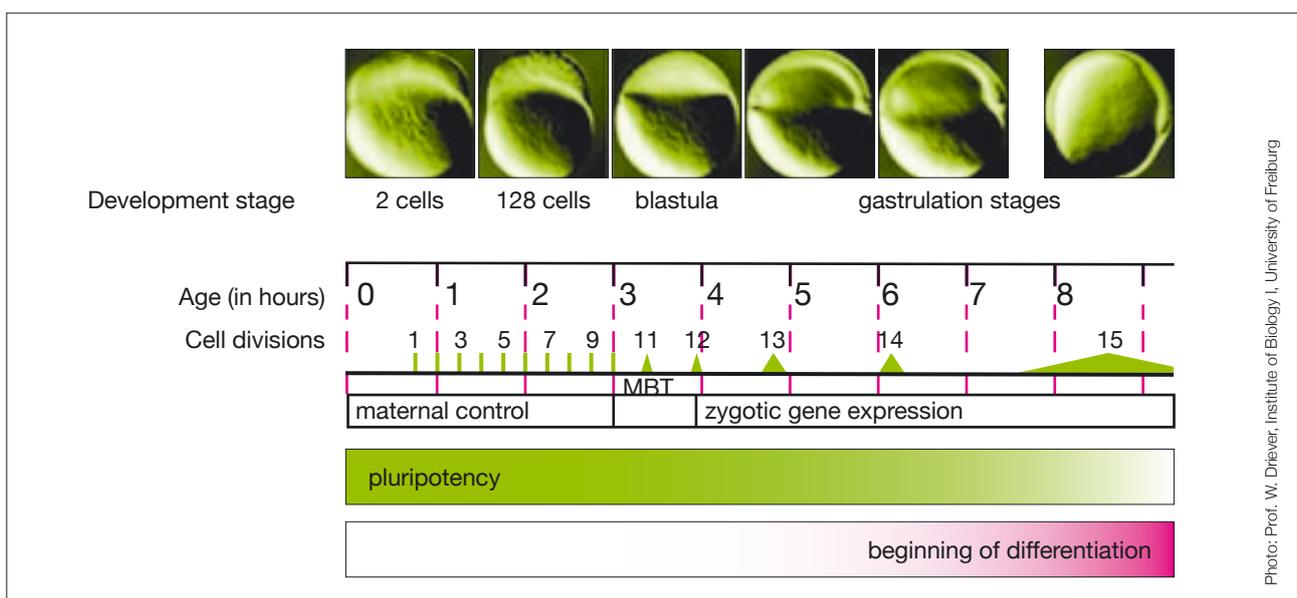
The manifold development potential of pluripotent stem cells, however, is cause for both hope and concern. How can we ensure that stem cells develop into stable cells of the type required, but not into tumours? To obtain this kind of security we need to have a better understanding of the regulatory mechanisms that control the natural differentiation of stem cells into embryonic tissue. Networks of signals and gene regulators control this differentiation in a step-by-step process. These differentiation steps are hard to investigate. In cell cultures they run asynchronously, and in mammalian embryos the relevant developmental stages are difficult to examine experimentally.

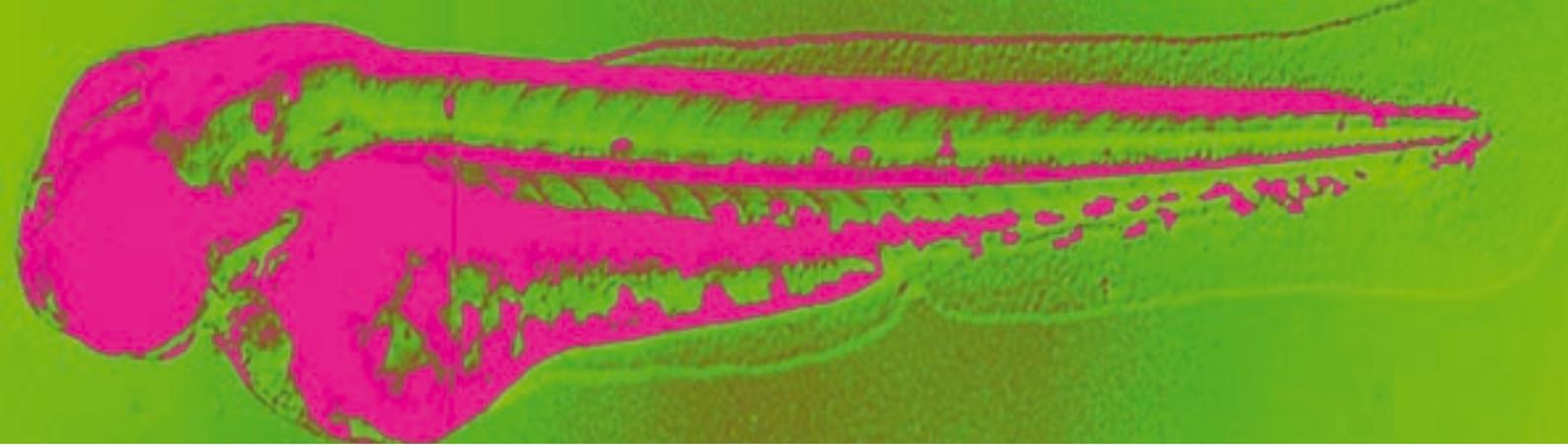
Synchronous development of hundreds of fish embryos

That is why we have chosen a different approach. By modelling the regulatory networks that play a part in regulating stem cell properties, we seek to understand the ways in which stem cells

Fig. 1: Zebrafish embryos

Zebrafish embryos develop synchronously. They are an ideal model system for research into regulatory mechanisms in the transition from pluripotent cells to fixed cell fates.





Two day old zebrafish larva – Photo: Prof. W. Driever, Institute of Biology I, University of Freiburg

differentiate. The model system used is the zebrafish, as large quantities of pluripotent cells can be extracted from its early developmental stages. The zebrafish has a further advantage – the synchronous development of several hundred embryos can be achieved in the laboratory, which means that precise, time-controlled data can be obtained for modelling.

Pou5f1, also known as Oct4, is a regulator of gene expression that plays a key role in maintaining pluripotency in the embryos of mammals and in creating new, induced pluripotent stem cells in cell culture experiments. In recent years several hundred genes have been identified that are regulated by Pou5f1 in the stem cell culture systems. One of the target genes of Pou5f1, the gene regulator Sox2, cooperates with Pou5f1 in regulating numerous other target genes. In mammalian systems these target genes include Klf2, Klf4 and Foxd3, which play complex roles in the embryo's further development. Due to limited opportunities for experimentation in mammalian systems, the properties of the early regulatory stem cell network are inadequately understood at present.

Evolution conserves regulatory network

Is the zebrafish a good model for the function of Pou5f1-dependent regulatory networks? We were able to show that Pou5f1 function is conserved in the embryo to an astonishing degree. Zebrafish embryos that do not create any functional Pou5f1 protein (maternal and zygotic MZ Pou5f1 mutant embryos, as they are called) during formation of the egg (maternal) and after fertilisation (zygotic) had their development fully rescued by an injection of mRNA encoding mouse Pou5f1 protein. This means that mammalian Pou5f1 performs the same function as fish protein in early embryonic regulatory networks.

In the zebrafish model we have identified the components of the regulatory network controlled by Pou5f1. Interestingly, despite an evolutionary distance of over 400 million years from the

common ancestors of bony fish and mammals, large parts of the Pou5f1 target gene network have been conserved: 120 (23%) of the 503 genes that are up-regulated by Pou5f1 in zebrafish are also dependent on Pou5f1 in mice. For the Pou5f1-dependent genes down-regulated in fish the percentage conserved is even higher at 152 (29%) out of 507 genes.

One mechanism for all vertebrates?

Time-resolved experiments in which embryonic gene expression was investigated at ten stages of development in wild types and in Pou5f1-deficient embryos were of crucial importance for getting a better understanding of the regulatory mechanisms. The period of time analysed covers stages ranging from early pluripotent cells to the period at which differentiation begins.

A bioinformatics investigation revealed the following findings: Along with the directly regulated genes, surprisingly almost a third of all genes expressed in the early zebrafish embryo are significantly changed in terms of their expression dynamics over the course of time.

A large group of differentiation genes, such as the eye control gene pax6, is expressed at a significantly earlier stage. Pou5f1 is therefore also responsible in the early embryo for the repression of differentiation genes.

A group of proteins that suppresses the expression of other genes – repressors – is activated directly by Pou5f1. As we were able to demonstrate, the expression of the repressor Her3 correlates to the repression of the differentiation gene pax6 for precursor cells

in the nervous system. We therefore postulate that the activation of specific repressors is a mechanism of the Pou5f1-dependent maintenance of pluripotency. For the Her3 control region, we were able to identify the DNA binding sites for Pou5f1 and Sox2 and explain the molecular mechanism controlling Her3 expression. We were also able to show that in mouse ES cells, too, Pou5f1 regulates the expression of Hes3, the gene that corresponds to zebrafish Her3. We therefore postulate that this mechanism is universally valid for vertebrates.

Interestingly, Pou5f1-dependent repressors are not expressed in all embryo cells but only in certain areas from which specific tissue classes (nervous system, skin, muscle/skeleton/internal organs) develop. We hope that thereby we have discovered a new mechanism for controlled differentiation of stem cells. Using experimental control of the expression of these tissue-specific repressors it ought to be possible to steer stem cells into a particular differentiation direction.

Network model provides new insights

For a detailed understanding of the regulatory properties, we have developed a small network model for a number of important components of the embryonic stem cell network (Fig. 2). Dependent on Pou5f1, more and more Sox gene activity (Sox2 und Sox11a) is built up in the early embryo. Two types of repressors of differentiation (RODs) are regulated: those that are only dependent on Pou5f1, such as Foxd3, and those that are controlled by Pou5f1 and Sox activity, such as Her3 and Hesx1. Individually or jointly, RODs control the promoters of differentiation (PODs), such as the orphan nuclear receptor Nr2f1 shown here as an example.

Can this model correctly predict the early development of embryos over time? To determine whether it can, the network model was mapped onto a system of differential equations. The parameters were optimised by means of the transcript quantities measured in the wild types (Fig. 2 black) and MZ-Pou5f1 mutant

Fig. 2:

(A) Simplified network model consisting of the stem cell factor Pou5f1 and the Sox gene regulators that jointly regulate repressors (RODs) and promoters (PODs) of differentiation.

(B) Modelled behaviour of the early stem cell network. Wild types correspond to black dots, fully Pou5f1/Oct4-deficient embryos to red dots, and maternally deficient Pou5f1 embryos to blue dots. The black and red graphs are the result of differential equations of the dynamic system. The dotted blue line shows the model forecast for maternally deficient Pou5f1 embryos. (Modified from Onichtchouk et al., 2010).

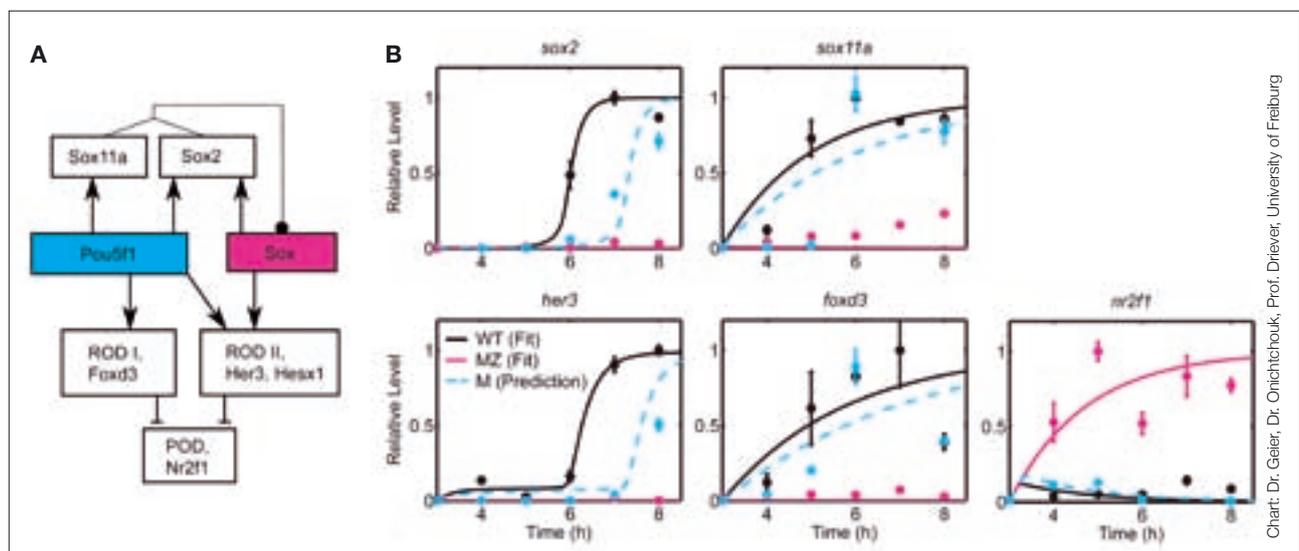


Chart: Dr. Geiler, Dr. Onichtchouk, Prof. Driever, University of Freiburg

embryos (Fig. 2 red). With this optimised model the network's temporal dynamics was then calculated only in maternal Pou5f1 mutant embryos (M; pale blue in Fig. 2). At the beginning of their development these embryos have no maternal Pou5f1 but then express Pou5f1 zygotically at a slow but increasing rate.

Finally, from the model we were able to infer the following predictions that are important for understanding the temporal dynamics of the stem cell network:

- Genes depending only on Pou5f1 have a single-phase expression dynamics with direct activation after the beginning of zygotic gene expression (see Fig. 2, FoxD3).
- Target genes that are dependent on both Pou5f1 and Sox2 have a two-phase activation with a weak early activation and a strong activation delayed by a few hours (see Fig. 2, Her3, Sox2).

Modelling revealed that the temporal dynamics of two-phase activated genes depends mainly on the temporal dynamics of the increase in Sox gene activity but not on changes in Pou5f1.

These findings provide us for the first time with a quantitative understanding of how the stem cell and early differentiation network in vertebrate embryos is controlled over time. The combination of embryology, bioinformatics and systems biology modelling has enabled us to gain an insight into not only the temporal dynamics but also the structure, function and evolution of the stem cell network.

Fig. 3 shows a simplified model of the stem cell network in zebrafish (A) and a postulated model for mammals (B) that is derived from the zebrafish findings. There are, of course, differences in evolution. In mammals the control circuit for maintain-

Fig. 3: Models of the early stem cell network organisation

In the zebrafish (A) and mouse embryo (B). See text for details. (Modified from Onichtchouk et al., 2010).

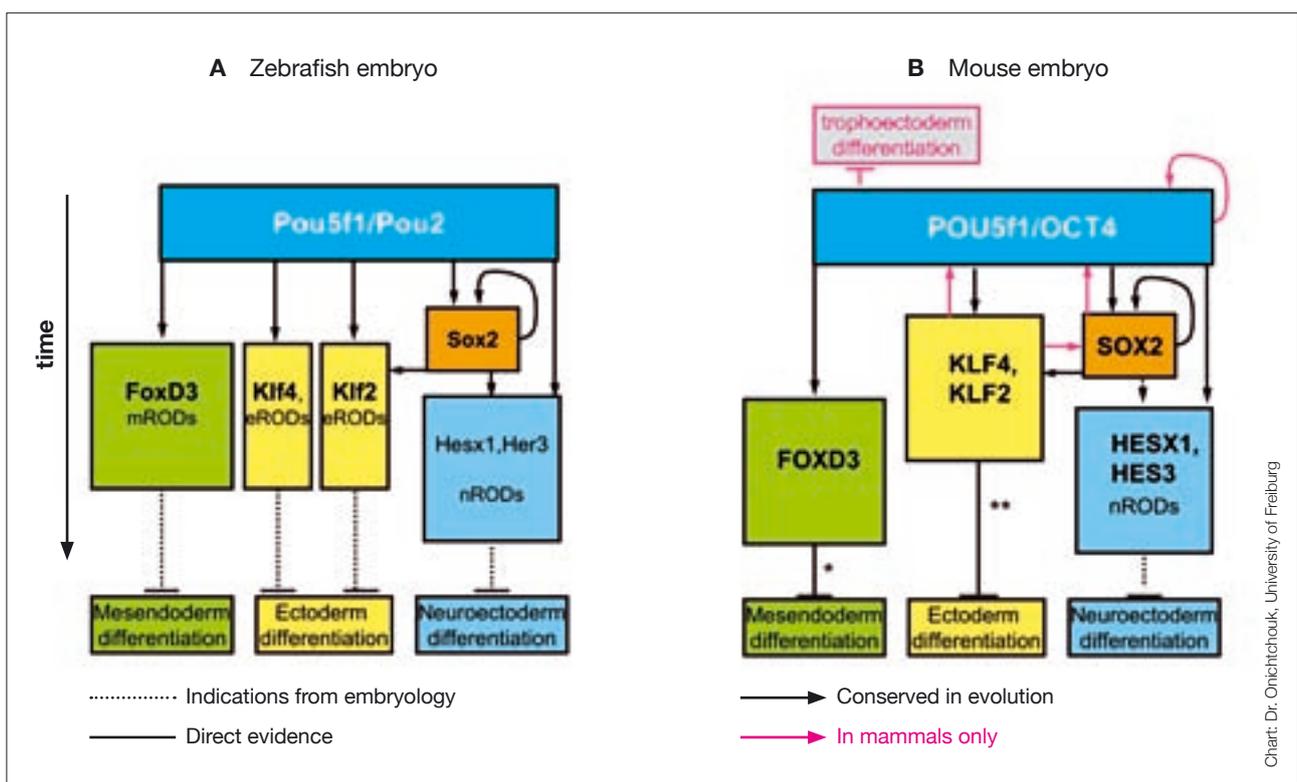
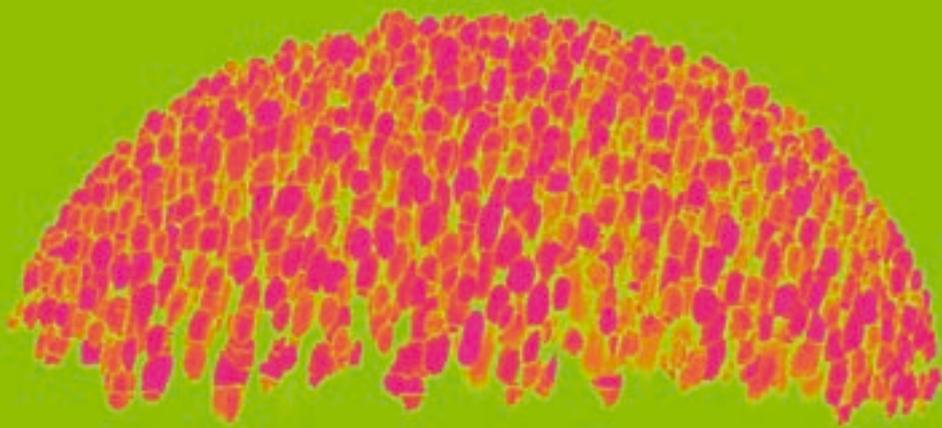


Chart: Dr. Onichtchouk, University of Freiburg



Cell nuclei of pluripotent cells in an early-stage zebrafish embryo – Photo: Prof. W. Driever, Institute of Biology I, University of Freiburg

ing pluripotency through the formation of Sox2-Pou5f1 auto-regulation and the inclusion of Klf s has developed in such a way that pluripotency is maintained over longer development phases. Important conserved elements, however, seem to be tissue-specific repressor systems that have merged into a single network in mammalian ES cells. The new evolutionary understanding of the stem cell network could make it possible to infer new paradigms for controlled differentiation of stem cells. That would be a major step forward for regenerative medicine.

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The research project in brief:

FRISYS – Freiburg Initiative for Systems Biology

www.frisys.biologie.uni-freiburg.de

FORSYS systems biology research units funded by the German Federal Ministry of Education and Research

www.forsys.net

Albert-Ludwigs-Universität Freiburg, Biosystems Analysis Centre (ZBSA)

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reliable information for pharmaceutical R&D decisions

Bayer Technology Services company profile

By Jörg Lippert

Bayer Technology Services GmbH (BTS) is an internationally operating Bayer subsidiary that provides fully-integrated solutions along the life cycle of chemical and pharmaceutical plants – from development to planning and construction to process optimisation at existing facilities. BTS also offers customised pharmaceutical development services.

These services form the core activities of the company's Systems Biology Competence Centre, which provides mathematical modelling tools such as PK-Sim® and MoBi® and carries out entire modelling and data analysis projects. The Centre is in charge of processing data, researching primary information, performing mathematical modelling, and simulation and compiling reports for licensing authorities.

The Systems Biology Competence Centre believes that its key function is to provide optimal information so that sound R&D decisions can be made. For clients of BTS this is hugely important. This is because developing and using new pharmacological therapies involves making decisions on a regular basis which have ethical, regulatory, medical, economic and practical dimensions that must be taken into consideration. Wrong decisions can have fatal consequences for individual patients or patient groups. In addition, substantial cost over-runs can often occur and, finally, the failure of a drug development programme can even pose a threat to a pharmaceutical company's continued existence. In the light of these issues, drug manufacturers are increasingly using mathematical models in their decision-making processes. Modelling and simulation are now taken for granted as tools that companies also use in statutory approval procedures for licensing authorities.

Impartial and reusable information

Up until now, most companies have been using modelling processes that are based on clinical data for individual drug candidates. These data-based approaches are, however, not really capable of incorporating substance-independent biological, pharmacological and medical knowledge that is available in published form or from internal sources. Even if studies of this kind contain drug-independent information, such as about patho-physiological patient attributes, this information is hard to extract and evaluate for other uses in the future.

BTS's Systems Biology Competence Centre overcomes the limitations of traditional data-based processes by using systems biology modelling methods. These methods can integrate expert knowledge, public and published information, and proprietary data from the client's preclinical and clinical research and development programmes and make this information available for the decision-making process. Consistent data preparation and mathematical formalisation ensure an impartial basis for decision-making. Substance-independent information can first be extracted from data that has been recorded for a specific drug candidate and can then be made available for current and future use.

Comprehensive project experience

The modelling experts at BTS have comprehensive experience in the use of systems biology methods. In the last five years they have successfully concluded over 100 projects with more than two dozen companies. Clients range from Top 5 pharmaceutical companies to small and mid-sized biotech firms. Projects have covered all phases and kinds of pharmaceutical decision-making processes, from matters regarding optimal targets and suitable targeting concepts, to selecting suitable lead candidates, evaluating in-licensing candidates, predicting doses for initial applications, designing and forecasting Phase III approval studies, right up to life cycle management for generic drugs.

These wide-ranging uses have been made possible thanks to sophisticated software tools such as PK-Sim[®] and MoBi[®], which are continuously being developed, as well as an accompanying systems biology research programme in which BTS benefits from cooperation with academic partners and research programmes funded by the German Federal Ministry of Education and Research (BMBF) and the EU. HepatoSys (Virtual Liver), QuantPro, the FORSYS partner programme and the D-Grid Initiative are all cases in point.

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systematic approaches to investigating the biogenesis of proteins

Focus on translation

By Gong Zhang and Zoya Ignatova

The 20 amino acids that occur in nature provide an inconceivably large number of combinations for unique protein sequences, especially when one considers that proteins consist of sequences of hundreds of amino acids. The physiological function of a protein depends, however, on its specific three-dimensional spatial structure. In addition, the role of each individual protein in the overall cell physiology is often largely determined by the amount of protein in a cell.

Our research work is aimed at obtaining a better understanding of the cellular protein balance and deals with question of how cellular regulation of this homeostasis is influenced by external factors (environmental conditions). We seek to answer this question with the aid of modelling techniques and innovative experimental approaches and technologies. Greater imbalances and disruptions in the homeostasis have the potential to reduce a cell's vitality with lasting effect and can also give rise to specific clinical pictures. This also applies to deviations from cell type-specific adjustment mechanisms and protein expression patterns that a cell uses to adapt to environmental changes.

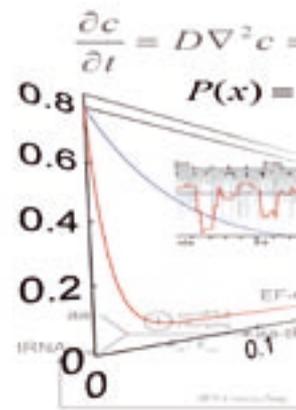
Examining silent mutations: co-variations in tRNA as a key regulator

DNA mutations that lead to changes in a protein's amino acid sequence can have harmful effects on its functionality. In contrast, the exchange of individual nucleotides, which do not lead to any changes in a protein's amino acid sequence due to the genetic code being degenerated, was long considered to be unproblematic. These mutations are known as silent or synonymous substitutions. There are, however, more and more indications that silent mutations may also lead to an increase in phenotypical variability. This effect can be explained by a change in splicing accuracy, a reduction in the protein translation pattern and a resulting structural and functional change in proteins. The importance that these processes have in certain circumstances is demonstrated by the fact that 40 different genetic diseases are now linked to silent mutations.

During translation – the synthesis of a protein in accordance with the genetic code on the mRNA – a cognate tRNA, charged with its specific amino acid, pairs off with the mRNA's triplet codon and the amino acid is linked to the growing polypeptide chain of the newly synthesised protein. The concentration of the different tRNAs can vary up to tenfold. This tRNA imbalance in relation to the number of triplets within an mRNA can lead to variations in the translation rate of each individual triplet. The rate of translation in an open reading frame is not necessarily uniform. Some mRNA areas are translated and converted into protein more slowly than others. These areas of slow translation typically follow a specific, non-coincidental pattern.

By using a broad spectrum of research approaches and bioinformatics analyses, we were able to show that the different speed of translation in individual mRNA areas clearly influences and facilitates processes that are downstream from the actual translation process. Clusters of slowly translated mRNA codons prefer to gather upstream of the boundaries of multi-domain proteins. The slow-translating regions actively co-ordinate the folding of the protein domains by providing them with the necessary time slot. Thus, a silent mutation in the coding region of a gene can, due to a resulting acceleration or deceleration of the translation process, lead to the synthesis of a wrongly folded – and therefore, no longer functional – protein.

As has been known for some time, the cellular tRNA content is modulated and regulated subject to environmental factors and environmental changes. Thus, the composition of the cellular tRNA pool reflects a cell's specific requirements for the synthesis of proteins at a given time and in given circumstances. We have now developed a model, with the aid of which we can predict and reliably calculate the dynamics of the protein synthesis process. We are thereby, gaining deeper insights into the correlation between the speed of mRNA translation and specific environmental conditions.



It can be concluded, that fluctuations in the concentration of charged aminoacylated-tRNAs can evidently also lead to a transient stalling of the ribosomes during the translation process. On the basis of our model the stress-related changes in tRNA availability thus lead to a premature termination of the translation and dissociation of the ribosomes. Alternatively, a transient, high need for a single tRNA species that the cell cannot meet could induce a frame shift and a premature termination of the translation process.

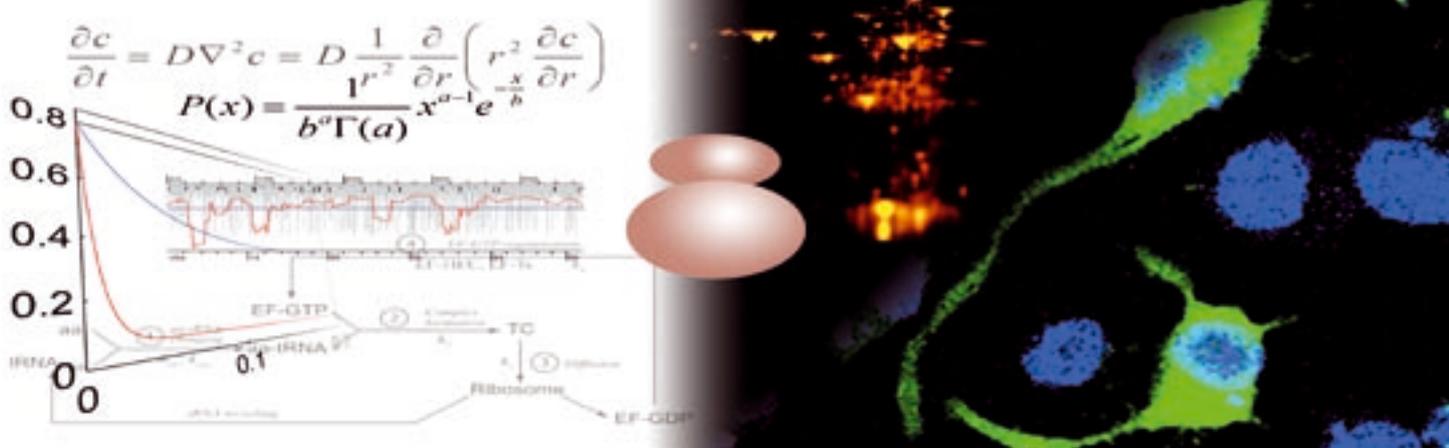
As the concentration of charged tRNAs is known to influence the local translation rate, it is likely that it can regulate the efficiency of translation and the cellular protein quantity. In order to be able to investigate the function of tRNAs in regulating a proteome (the totality of proteins in a cell), it is necessary to determine the quantity of each individual tRNA species in the cell in different conditions. The genetic tRNA pool of *Escherichia coli* bacteria comprises 50 tRNAs, that of a mouse is 249, and that of a human is more than 400 tRNAs. For the quantitative evaluation of the cellular tRNA pool, we use various methods. They included traditional approaches (2D electrophoresis, HPLC) and newer high-resolution methods (microarrays, deep sequencing, and quantitative RT-PCR). In their entirety these new experimental approaches have the potential to provide us with a systematic insight into the changes in the tRNA pools and their effect on a cell's proteome.

A systemic examination of protein aggregation

In a large number of neuropathological diseases, including Huntington's and various ataxias, the corresponding protein forms insoluble fibrillar amyloids and plaques. Against this background, our group's research focus is on what are known as polyglutamine diseases. In neuropathology they are blamed for at least nine different diseases. A striking characteristic of polyglutamines (polyglutamine proteins), whose primary sequence in some cases differs substantially from that of other human proteins, is the presence of a labile homopolymeric polyglutamine area with up to 30 or 40 residual glutamines. The length and number of repetitions within the protein is in reverse proportion

to the severity of the disease and the patient's age at the time of its onset. By means of a combination of *in vitro* and *in vivo* methods with complex and single molecular resolution we were able to prove that the aggregation of proteins containing polyglutamine is a multi-stage process in the course of which mature fibrillae are formed from different aggregates. The composition and toxicity of the aggregates differ, and the dynamics of the entire process are influenced by the sequences that flank the polyglutamines and by the cellular environment. With the aid of dynamic molecular simulation, we are able to pursue conformation changes and grouping events that are invisible using experimental methods.

Starting with the assumption that in principle any kind of external stress can lead to fluctuations in cell volume and in concentration of various molecules in the cytosol, it is conceivable that environmental influences may give rise to a reduced stability in cellular protein mass and to an accelerated protein aggregation rate. To counteract the different physiological stresses that pose a threat to protein stability and function, cells synthesise and accumulate a large number of small organic molecules known as osmolytes or chemical chaperones. These chemical chaperones are able to change the intracellular environment to such an extent that the conditions for the aggregation of polyglutamines change too, as we were able to show in wide-ranging experiments *in vivo* and *in vitro* using biochemical, cell biology and high-resolution spectroscopic approaches (FCS = fluorescence correlation spectroscopy, FRAP = fluorescence recovery after photobleaching). The osmolytes trimethylamine N-oxide (TMAO) and proline, for example, transfer the formation of amyloid fibrillogenesis of the pathological Huntington Exon 1 fragment to non-amyloidogenic amorphous protein accumulations by means of two different molecular mechanisms. While the strongly solvophobic effect of TMAO leads to a formation of amorphous protein aggregates with minimally exposed surfaces, proline stabilises the monomer protein and suppresses an accumulation of early transient protein aggregates. Glycine betaine, in contrast, intensifies the fibrillogenesis of polyglutamines by means of a mechanism reminiscent of the process of functional amyloid formation.



A systems biology research approach to understanding the biogenesis of proteins (Photo: Zoya Ignatova, University of Potsdam)

Remarkably, none of the native osmolytes characterised to date is able to fully suppress the formation of protein aggregates. Indisputably, however, osmolytes redirect amyloidogenesis to alternative routes that lead to the formation of protein aggregates with a lower toxicity.

Using a broad spectrum of experimental approaches combined with modelling approaches, bioinformatics methods and dynamic molecular simulations, we were able to develop a systematic plan of the strongly heterogeneous aggregation process and its specific kinetics.

The research project in brief:

Project name: „SysMO: Systems Biology of Microorganisms
SysMO is a transnational initiative to promote research that is funded by the German Federal Ministry of Education and Research (BMBF) together with ministries and research organisations in Austria, the Netherlands, Norway, Spain and the UK. KOSMOBAC is a SysMO sub-project that investigates ion and solute homeostasis in enterobacteria.

Associated partners: University of Aberdeen: Department of Biological Medicine, Prof. Ian Booth, Dr. Samantha Miller, Dr. Celso Grebogi, Dr. Alessandro de Moura; University of Munich, Department of Microbiology, Prof. Dr. Kirsten Jung; University of Groningen: Department of Biochemistry, Prof. Bert Poolman; Instituto de Investigaciones Marinas IIM: Department of Bioprocess Engineering, Prof. Julio R. Banga; Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg: Dr. Andreas Kremling

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“theory needs experimental partners”

Interview with **Jana Wolf**

Since 2008 Jana Wolf has headed the Mathematical Modelling of Cellular Processes junior research team at the Max Delbrück Center for Molecular Medicine (MDC) in Berlin. Most research scientists at the MDC use sophisticated experiments and precise measurement procedures to shed light on the complex processes that occur within cells. Jana Wolf takes a very different approach. She tackles the complexity of living things using mathematics and computer simulation. Here, Jana Wolf talks to *systembiologie.de* about her work and creativity and the exciting dynamics of the life sciences.

Ms. Wolf, what prompted you to join the MDC?

My junior research team is funded by the FORSYS systems biology initiative and the Helmholtz Alliance. Under the terms of the funding, I could choose the research location. I decided on the MDC because the opportunities for cooperation here are excellent for a group that is engaged in theoretical work – because theory needs experimental partners. Our main cooperation partner at the MDC is Claus Scheiderei's team. We also cooperate with a number of other MDC groups and with teams from other institutes.

Mathematical modelling and empirical, experimental research into life processes are two very different approaches. How do the two find common ground?

The MDC provides a highly stimulating environment. There are many contacts because our theoretical group is right in the middle of excellent experimental teams. We start talking over lunch or a cup of coffee, and colleagues who focus on experimentation now contact me directly because, for instance, their results are contradictory or confusing and they cannot make any further headway with a purely experimental approach. They then consider whether a model might help them to do so.

Are reservations about mathematical methods and models not widespread among laboratory biologists?

That is changing. More and more quantitative and system-wide data are being collected that can no longer be explained solely by means of verbal argumentation. That is why mathematical modelling is increasingly seen as a very valuable aid.

To which biological issues are you applying your mathematical tools?

Our focus is the signal transduction and the regulation of gene activity in cells. Signals reach the cells from outside. They are then processed and activate or deactivate the expression of genes in the cell nucleus. Signal transfer does not occur in a linear chain, however, but rather in a network with many nodes and regulatory mechanisms. One of these nodes is the transcription factor NF- κ B, which is important for immune response and embryonic development and also plays a role in the development of cardiac and circulatory diseases and Hodgkin's lymphoma. Our aim is to understand how this complex signalling network generates the resulting dynamics and how disturbances in the signalling pathways cause diseases.

“The interdisciplinary approach fascinated me. I have never worked any other way.”

What does your mathematical toolkit look like?

The most important method is mechanistic modelling and simulation. We map the molecular players in the cells and their temporal changes in concentration onto differential equation systems. So we translate hypotheses about cellular processes into a mathematical model. If the model then shows similar responses to perturbations as those observed by colleagues in the laboratory, we regard that as an indication that our hypotheses are correct. We also carry out bifurcation and sensitivity analyses. They enable us to determine the impact of individual processes on the overall behaviour.



Dr. Jana Wolf (Photo: David Ausserhofer, MDC Berlin)

At what point do you find that mechanistic modelling has reached its limitations?

It is difficult to apply it to very large signalling and regulatory networks. In this case it is necessary to describe sub-systems first and link those to the overall network. An alternative approach for large networks is logical modelling, which describes the logic of interactions in the network in a qualitative way. We aim to make greater use of this approach in the future.

What focal points and trends do you see in systems biology research?

There is an exciting dynamics in biology since the quantity and quality of data is constantly improving. This can be seen, for example, at the new Berlin Institute for Medical Systems Biology, which is run jointly by the MDC and Charité. There, powerful mass spectrometric methods and high-throughput sequencing are being used to record the proteome and genome with high accuracy. That will provide us with a comprehensive data foundation to advance our biological understanding. The specificity of cells is another issue that is increasingly coming to the fore in systems biology research. The signalling and regulatory networks are evidently not the same in all cells, even for fundamental processes. There are differences between tissue types and between healthy and unhealthy conditions. Even within a given tissue type cells can show a marked range of regulatory patterns. We need a deeper understanding of this cell specificity to better develop targeted and effective therapies to treat, for instance, cancer or neurodegenerative diseases.

Can modelling help to solve these issues?

To understand cell specificity we have to explore the network composition and regulatory complexity of signal transduction, gene regulation, and metabolic processes in different cell types. It is not sufficient to describe these process levels separately, one must also gain an integrative view of how they interact. For these detailed and quantitative descriptions and an overall understanding of the interaction within large networks, we need mathematical models.

How did you find your way into theoretical biology and mathematical modelling?

I always had a keen interest in mathematics and biological processes. The interdisciplinary approach also fascinated me. I have never worked any other way. Interdisciplinary research is very interesting because you meet people from different fields of research. I studied biophysics, a course of study which combines the main biological subjects with mathematics and physics. I learnt mathematical modelling during my studies and my doctoral thesis work under Professor Reinhart Heinrich at Humboldt University in Berlin. Working in his group was a very valuable experience. In my post-doc period I also worked in the R&D department of a drug company. I found it very exciting to see how mathematical modelling is used there to predict the effectiveness of drugs and to optimise production processes.

What do you need in order to stay creative?

A good environment, stimulating discussions, interesting conversational partners, and a good cup of coffee. Ideas develop when people with different vantage points and different backgrounds talk with each other. The MDC is perfect for that. Berlin, too, with its diverse scientific community, is very stimulating. What's more, I can combine my personal life with my scientific work here.

The interviewer was Thomas Früh.

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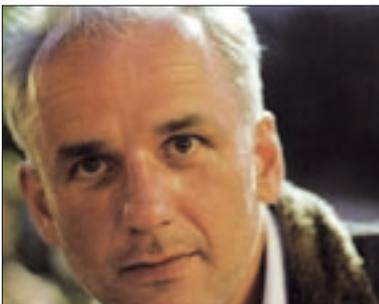
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Berlin Science Award for Systems Biologist Nikolaus Rajewsky

Nikolaus Rajewsky of the Max Delbrück Center for Molecular Medicine (MDC) in Berlin-Buch has been awarded the Science Award of the Governing Mayor of Berlin for his “outstanding research work, the implementation of which helps to solve problems in science and society.” The award, endowed with € 40,000, was presented at the inaugural event of the Year of Science 2010. Nikolaus Rajewsky is Professor of Systems Biology at the MDC and Charité in Berlin-Buch and Head of the Berlin Institute for Medical Systems Biology (BIMSB). In 2008 he has also been appointed Global Distinguished Professor of Biology at New York University. Professor Rajewsky carries out research on the function of microRNAs, small ribonucleic acid molecules, and was able to show that a single microRNA can control the creation of several hundred different proteins. In doing so, he confirmed that microRNAs participate in regulation of nearly all important life processes in cells and organisms. With the aid of microRNAs, disease-specific processes can be explained and, in the future, may possibly be treated, too.

Source: MDC Press Release



Professor Nikolaus Rajewsky (Photo: private)

DKFZ and Life Technologies Corporation establish Germany's largest sequencing centre

In June 2010 the German Cancer Research Center (DKFZ) and US-based Life Technologies Corp. announced the establishment of a national centre for high-throughput sequencing in Heidelberg. Once it has been set up, it will be the largest sequencing facility in Germany and Europe's first national sequencing centre dedicated to systems biology. The centre is jointly supported by Life Technologies, the DKFZ, and the German Federal Ministry of Education and Research. When fully equipped in early 2011, the centre will operate ten SOLID™ 4 hq systems. Taken together,

the facility will be able to sequence over approx. 750 individual human genomes per year. Within the center, research will focus on establishing next generation sequencing methods for systems biology methods, which require large numbers of extremely precise measurements. Furthermore, one of the center's first objectives will be the sequencing of the genomes of more than 600 samples from early childhood brain tumours within the Ped-BrainTumor project, Germany's first national contribution to the International Cancer Genome Consortium (ICGC).

Source: Press Releases DKFZ and Life Technologies

Federal Minister announces two new German Contributions to the International Cancer Genome Consortium

Within the International Cancer Genome Consortium (ICGC) scientists world-wide are working together to decipher the genomes of 50 different cancers. In June 2010, the German Federal Minister of Research and Education, Prof. Annette Schavan announced that Germany will invest 15 million Euros over a period of five years to finance two further German projects within the ICGC. The new projects focus on prostate cancer and malignant lymphoma, two major cancer entities. By completely sequencing genomes of tumour cells from several hundred patients and accompanying investigations, ICGC-researchers are aiming to detect the relevant mutations for each cancer type and to develop individualized therapies. Together with the first German contribution to the ICGC focusing on early childhood brain tumours, which started in early 2010, the German ICGC-contribution now becomes increasingly relevant. The project on prostate cancer, the most common cancer in males, is carried out by the German Cancer Research Center (DKFZ), the University of Hamburg-Eppendorf, and the Martini-Klinik Eppendorf. The aim of the project coordinated by Dr. Holger Sültmann (DKFZ) is the detection of specific mutations that are causative of prostate cancer in order to develop novel individual therapies. The second newly announced project will analyze the cancer genomes of malignant non-Hodgkin's lymphomas. These cancers affect the lymphatic system and are among the most frequent cancers in both childhood and adulthood. This project will be coordinated by Prof. Dr. Reiner Siebert (University of Kiel).

Source: BMBF press release

Using Systems Biology to Understand the Formation of Blood

After loss of blood our body responds with an increased production of red blood cells (erythrocytes). Activation of the blood-forming cells in the bone marrow is initiated by the hormone Erythropoietin (Epo) that is synthesized in the kidney. The group of Ursula Klingmüller (DKFZ Heidelberg) and Jens Timmer (University of Freiburg) recently published in *Science* how a fast throughput of Epo receptor molecules on blood-forming cells ensures that our body can respond even to extreme increases of Epo levels with an adequate supply of red blood cells. The researchers combined experimental data with mathematical models in a systems biology approach. Thereby, they were able to show that after binding of Epo to its receptor both molecules are rapidly taken up into the interior of the cells where they are degraded. During the process, the cell surface is continuously equipped with newly synthesized receptor molecules that are supplied from intracellular storage places. This fast re-supply ensures continuous responsiveness of the cells for new incoming signals. Genetically produced Epo is an important treatment for patients suffering from low counts of red blood cells (anaemia). The results of the scientists from Heidelberg and Freiburg could promote the development of Epo variants with enhanced binding properties and thus increase the effectiveness of anaemia treatment.

Source: Press Release DKFZ;

Original Publication: Becker, V., M. Schilling, et al. (2010). "Covering a broad dynamic range: information processing at the erythropoietin receptor."

Science 328(5984): 1404-8. DOI: 10.1126/science.1184913

Analytica Research Award for Dr. Matthias Selbach of the MDC – New method developed for measuring the production of thousands of proteins

The biologist Dr. Matthias Selbach of the Max Delbrück Center for Molecular Medicine in Berlin-Buch has been honored with the 2010 Analytica Research Award. Dr. Selbach was awarded the € 25,000 prize "for his work on the influence of microRNAs on the protein production of cancer cells". The prize is granted by the Gesellschaft für Biochemie und Molekularbiologie e. V., with the pharmaceutical company Roche donating the prize money. Dr. Selbach and his colleagues were commended for developing a new method by which the production of thousands of proteins can be measured simultaneously. In collaboration with Professor Nikolaus Rajewsky's group at the MDC, the method has already

been put to successful application. Dr. Selbach and his group also demonstrated that a single microRNA can control the creation of hundreds of proteins. As there are microRNAs active in cancer cells that are not found in healthy cells, microRNAs are considered to be promising candidates for diagnostics and therapy.

Source: MDC Press Release



Dr. Matthias Selbach (Photo: David Ausserhofer/MDC)

EU funding for PathoSys: new Algorithms for Host-Pathogen Systems Biology

Many pathogens are prime examples to underline the need for systems biology approaches, since the intricate interplay for example between a virus and the host cell cannot be understood by studying the pathogen alone. Scientists from eight academic and two industrial partners have now joined forces to develop new systems biology approaches to study pathogen-host interactions. Funded with a sum of € 3 million for 4 years by the European Union in the 7th Framework Program, the *PathoSys* project focuses particularly on Hepatitis-C virus (HCV), the causative agent of Hepatitis-C. "Our ultimate aim is to develop new treatment strategies using a systems biology approach", says Lars Kaderali, who is coordinating the *PathoSys* project jointly with Roland Eils at the BioQuant centre of Heidelberg University. To achieve its objectives, *PathoSys* will develop novel mathematical algorithms for model development, model fitting and model analysis, and will integrate data-driven and knowledge-driven approaches with the latest laboratory techniques in HCV biology. *PathoSys* is particularly thought to tighten the collaboration between EU countries and Russia in the field of systems biology. It combines research groups from Russia with partners in France, Cyprus, Turkey, Israel, and Germany.

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events

Conference Report SBHD 2010

International Conference on Systems Biology of Human Disease 2010 – a new platform for exchange between the US and Germany

Boston, June 16-18, 2010

The International Conference on Systems Biology of Human Disease 2010 was a three-day conference focusing on mammalian systems biology, particularly as it applies to human disease and therapy. The conference was organized by the Council for Systems Biology in Boston (CSB²; www.csb2.org) supported by the MIT/HMS-based Centre for Cell Decision Processes and the Centre for Cancer Systems Biology at the Dana-Farber Cancer Institute. As a novelty, the organizers teamed up with the two largest systems biology initiatives in Germany, FORSYS-Research Centers for Systems Biology (www.forsys.net) and the Helmholtz Alliance on Systems Biology (www.helmholtz.de/systemsbiology) as well as the BioQuant Centre in Heidelberg (www.bioquant.uni-heidelberg.de). Due to the generous support of the German Federal Ministry of Education and Research (BMBF), 40 graduate students and postdoctoral fellows were able to attend the conference. In future, the conference will alternate between Boston and Heidelberg in order to create a bi-national exchange platform between Germany and the US.

The major focus of the conference was on computational biology, protein networks, and systems pharmacology. More than 200 participants presented recent research results in talks and on 80 posters. During the conference, Markus Covert from Stanford University received the CSB² Price in Computational Biology for his work on analyzing the heterogeneity of cellular responses after external stimulation and Melissa Kemp (Georgia Tech & Emory University) received the CSB² Price in Systems Biology for her work on the role of thiol oxidation in signal transduction.

The International Conference on Systems Biology 2011 will take place once again at the Harvard Medical School in Boston on June 22-24, 2011 before it moves to Heidelberg in 2012.

Conference Report SBMC 2010

A Window to New Dimensions – Systems Biologists meet in Freiburg

Freiburg, June 3-5, 2010

During the Conference on Systems Biology of Mammalian Cells (SBMC), 350 scientists came together to discuss cutting-edge research findings, trends, and visions in the field of systems biology of mammalian cells.

The conference was organized by the German Virtual Liver Network, the German Systems Biology competence network for the study of the liver. It took place from June 3-5, 2010 in Freiburg under the patronage of the German Federal Minister for Education and Research, Prof. Dr. Annette Schavan.

In his welcome speech, Dr. Helge Braun, Parliamentary State Secretary at the Federal Ministry for Education and Research (BMBF), emphasized the central idea of systems biology: “By combining biomolecular approaches with mathematical computer models, systems biological research creates new solutions for an individualized medicine, with benefits for every patient.”

In a broader sense, one of the pioneers in the field of systems biology, Prof. Denis Noble from the University of Oxford, re-emphasized this point by pointing out the fundamental necessity of intensive basic research in order to understand the manifold, complex processes of life on all levels ranging from the genome to the entire organism. In his talk, Noble drew a comparison to the great cathedrals of Europe, the construction of which usually extended over a period of several centuries. “Today’s science – including systems biology – is considered the cathedral of the 21st century. And just as in the case of those impressive religious buildings, the field of systems biology needs a long and intensive construction period in order to arrive at long-term results”.

Following this spirit, mathematicians, biologists, and physicists convincingly illustrated their overall goals to extend systems models onto the cellular level towards a holistic approach to include the whole organ or even a complete organism.

The organizing systems biology initiative, the German Virtual Liver Network, was established in 2010. It is built on the work of HepatoSys, the first German systems biology initiative that

was set up in 2004 by the BMBF to do research on intracellular processes in liver cells (hepatocytes). The German Virtual Liver Network continues this work to gain an understanding of hepatocytes at an even higher level of complexity to generate a dynamic view of liver functions.

Save the date: Germany to host the International Conference on Systems Biology (ICSB) in 2011

12th International Conference on Systems Biology, August 28th - September 1st, 2011 in Heidelberg / Mannheim

For the second time after 2004, Germany will host the ICSB 2011, the world-largest systems biology event. As the annual meeting of the International Society for Systems Biology (ISSB), the ICSB is the premier meeting for presentation of the hottest topics in this vivid new field of research. Furthermore, it aims at helping researchers to establish and coordinate alliances, cooperations, and scientific exchange in international systems biology research.



The ICSB 2011 will be held at the Conference Centre Rosengarten in Mannheim near Heidelberg. Its major topics will comprise all major subjects in systems biology research and will specifically focus on systems biology in medical and industrial applications as well covering related fields such as synthetic biology. Before and after the conference, satellite symposia will be held in Heidelberg, addressing topics like ethical and societal implications of systems biology. Contributions to set up sessions and satellite meetings are welcomed!

Further information and contact are available at

www.icsb-2011.net

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about us

Presenting the systembiologie.de editorial team

systembiologie.de would like to make the successes of German systems biology accessible to a wider public in an illustrative way. The magazine, which will initially be published twice a year in German, is produced jointly by the offices of the nationwide systems biology networks Helmholtz Alliance on Systems Biology, FORSYS – Research Units for Systems Biology, Virtual Liver Network, and Project Management Jülich (PtJ). It is financed by

the Helmholtz Alliance on Systems Biology, which receives funding from the Helmholtz Association's Initiative and Networking Fund, and by the German Federal Ministry of Education and Research. By compiling translated articles from the German magazine into an international edition, we will present the results to a worldwide audience.

The editorial team of systembiologie.de's first international issue (from left to right)

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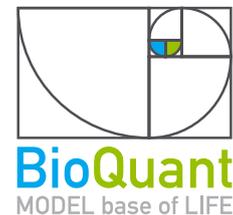
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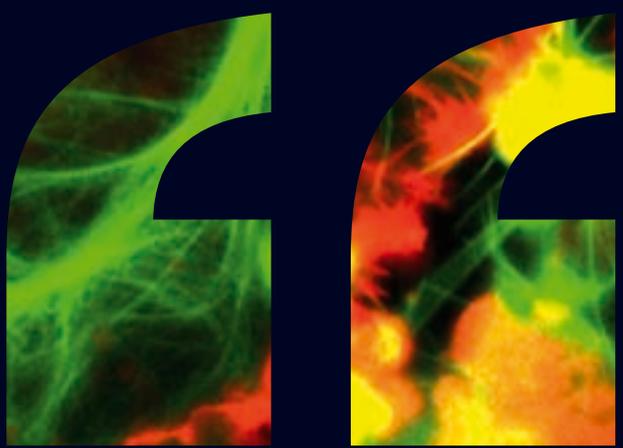
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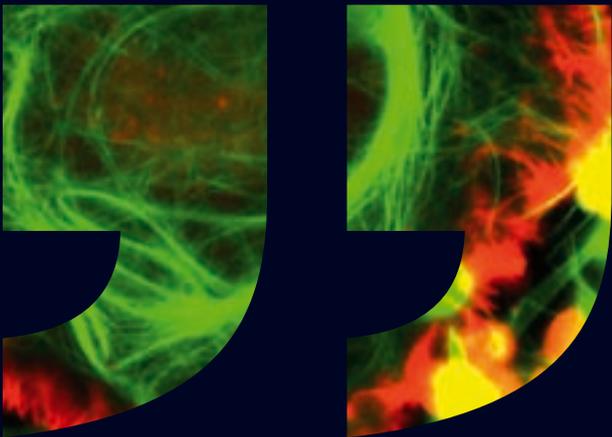
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