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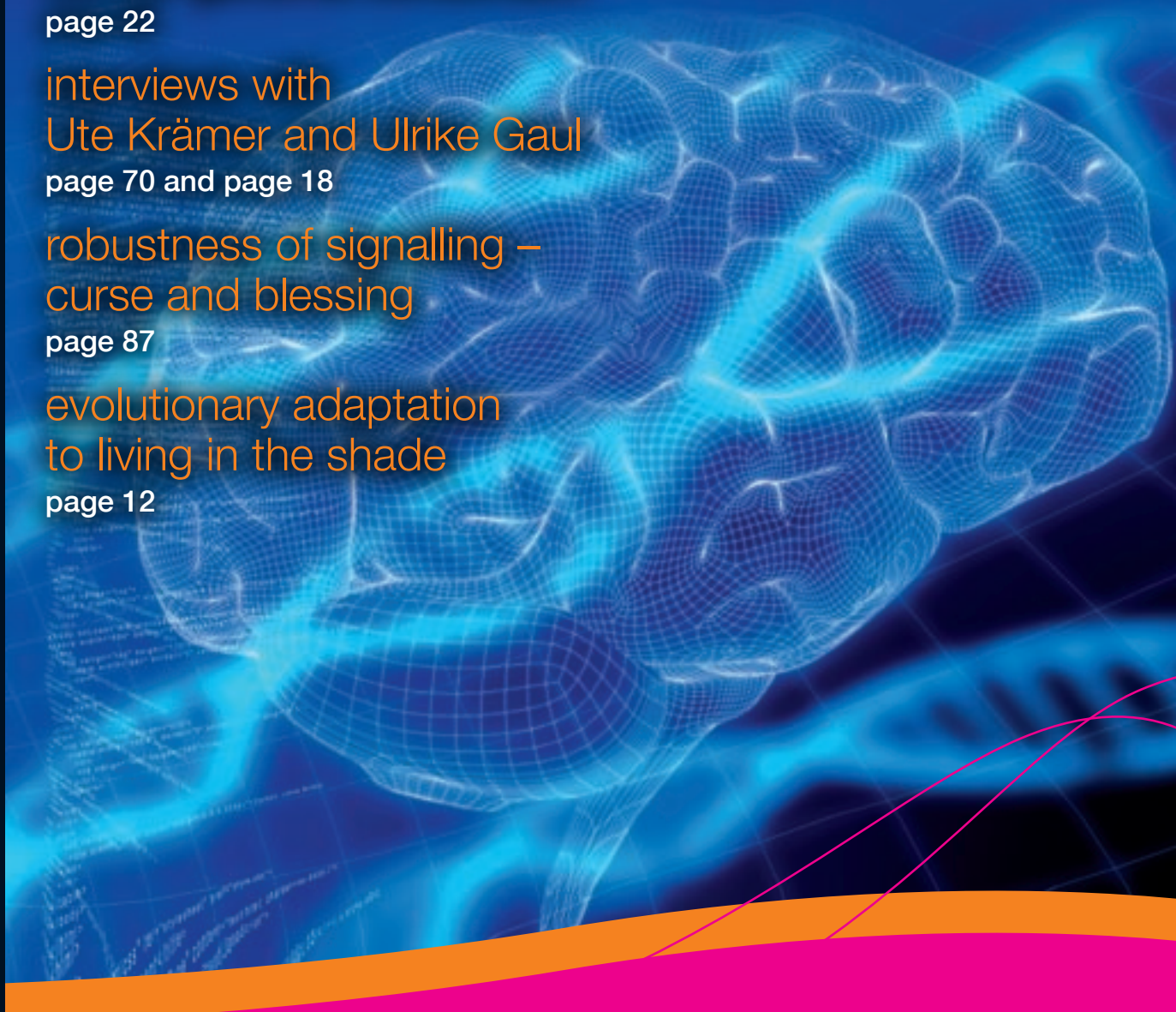
from page 22



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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 modelling, 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.

welcome note

Dear Reader,

Our insight into basic biological systems has increased tremendously in recent decades. This means that we now have more possibilities for diagnosing and treating diseases. At the same time, however, we are also facing new challenges. The psychological and physical stress of modern-day life, age-related diseases and the provision of a universal system of affordable health care are just a few examples.

The Federal Ministry of Education and Research (BMBF) is tackling these challenges under its Health Research Framework Programme. We are establishing clear priorities and defining objectives for the years to come. A key element of the strategy is the establishment of six German Centres for Health Research, which are devoted to the major common diseases and will transfer progress to the patient. A further focus is the field of personalised medicine.

We are adopting a systems biology approach towards personalised medicine and research into common diseases. The great complexity of living systems must be taken into account when exploring fundamental disease mechanisms, identifying molecular switching points and preparing new diagnostic agents. Systems biology is an appropriate instrument in this context and is therefore an important component of the Framework Programme in the Life Sciences.

I am therefore all the more delighted that the fourth edition of systembiologie.de is dealing in detail with the link between medicine and systems biology.

I hope that you will find this magazine stimulating reading and that it will provide a host of new insights into this fascinating field of research.

Prof. Dr. Annette Schavan, Member of the German Bundestag
Federal Minister of Education and Research

welcome note

Dear Reader,

Innovative technologies are increasingly becoming a driving force of health research. Scientists are developing new methods to generate detailed datasets of the molecular inventory of individual cells and of the complex interplay between these molecules. These data form the ideal foundation for systems biology models and analyses that can be used to gain an even better understanding of cellular growth and the differentiation processes of cellular compounds right through to organs.

Prime examples of recent developments in this field are the new genome sequencing techniques that have led to a large number of innovative approaches in health research. While it took more than ten years at a cost of several billion dollars to sequence the first human genome, today a person's entire genome can be sequenced in a few days and for just a few thousand euros. As a result, it is now possible to individually search for genetic causes and contributing factors in the development of disease and chronic disease. For example, it is becoming clear that the emergence and progress of cancer can be highly individual in each patient. Projects such as the International Cancer Genome Consortium (ICGC) are sequencing the genomes of hundreds of cancer patients, thereby creating the scientific basis for identifying new disease-relevant gene variants.

In major projects like this, analysing and managing colossal amounts of data is a big challenge that should not be underestimated. To cope with this challenge, close cooperation between researchers in different disciplines is essential. For instance biomedical research can benefit from experience in particle physics, where scientists have a long-standing experience in handling immense amounts of data. With its broad research portfolio, the Helmholtz Association provides an ideal basis for supporting interdisciplinary research of this kind. Therefore, questions that cut across research fields will play an increasing role in the Helmholtz Association in the future.

In the current issue of systembiologie.de, standout examples are used to describe how medicine is already benefiting from the findings of basic research. It accelerates the progress of new research findings into clinical application and enables new or improved methods of prevention, diagnosis and therapy to benefit the patient sooner.

I hope every reader will find the fourth issue of systembiologie.de to be an exciting read.

Yours, Jürgen Mlynek

President of the Helmholtz Association

foreword

Dear Reader,

"Actually I was the first Green, I have always been singing about meadows and forests."

Just as German folk singer Heino interprets his political ethos, after only a decade of systems biology research an ever-growing proportion of life scientists appear to see themselves as a fundamental part of systems biology. Perhaps the fact that it triggered a rethink should be celebrated as systems biology's greatest achievement. The life sciences have certainly managed the transition from a primarily qualitative, descriptive science to an increasingly quantitative, mechanically thinking one. The mathematisation of life sciences is forging ahead continuously. There are notable scientific contributions in all research fields that would not have been possible without a systems biology approach.

So is it time to sign off from systems biology and find a new hot topic and another trend? Certainly not! Although the zeitgeist in the research policy landscape calls for new topics, scientific reason tells us to concentrate on strengths as we look forward, to integrate things that have been achieved into the design for the transition of systems biology into the next decade.

An explicit, early objective of systems biology was to make an enduring contribution to medical research. However, in the early stages the science was, naturally, oriented entirely towards basic principles. Many efforts were made to gain a basic understanding of molecular and cellular mechanisms that were found to be consistent with diseases as diverse as cancer and neurodegenerative conditions and were described in terms of systems biology. This laid the foundation stone for taking the first step in the transition from *systems biology* which was predominantly oriented towards basic principles to mainly application-related *systems medicine*.

So this is a new hot topic after all? Hardly. Rather, we can and must try to take a fundamentally new look at causal molecular and cellular disease processes. This type of approach needs systems biology and is highly unlikely to come from genome research alone. Systems biologists, for their part, want to make a significant contribution to health research, and I would venture to assert that no more than ten years from now systems medicine methods will be an indispensable part of basic patient care.

"I cannot say whether things will get better if we change; what I can say is they must change if they are to get better," proclaimed the German aphorist and physicist Georg Christoph Lichtenberg (1742–99). Even though the promised benefits of genome research have been only incompletely delivered in recent decades, through the combined efforts of genome research, systems biology and clinical research, we now have a unique opportunity to achieve truly significant progress in our understanding of the molecular and cellular processes underlying the most common diseases and thus ultimately to succeed in redesigning diagnosis and treatment. This may be a bold aspiration, but it is a challenge to which the research community of systems biologists will gladly rise.

I would like to wish you a pleasurable insight into the world of systems medicine.

Yours, Roland Eils

Editor in Chief

index

digital and analogue data processing in the p53 signalling pathway

Using fluorescent reporters to track a tumour suppressor

by Alexander Loewer

The cells in our bodies have the intriguing capacity to react flexibly to changes. They do this by detecting signals from the environment, linking them to information about their internal state and triggering the appropriate response. This cellular signal processing is mediated by complex molecular networks with hundreds of individual components. The major nodes and edges of these networks have been identified by now. But how do they function in living cells? How are signals encoded, processed and decoded again? How do cells distinguish significant signals and unavoidable background noise? These questions hold the key for making selective manipulations of cell behaviour possible, a basic prerequisite for developing effective therapies.

Cells react to external and internal stress signals

Stress is a permanent feature of life. In our everyday lives, we constantly strive to strike a balance between the conflicting priorities of work, family and leisure. Similarly, the cells in our bodies have to defend the integrity of their molecular components from stress that both assails them from the environment and is a consequence of processes essential to life. In order to understand the basic principles of dynamic signal processing, we study the cellular response to stress. For example, solar radiation damages the genetic make-up of our skin cells, while harmful radicals are created by the production of energy during cellular respiration. Our cells have to detect this stress and initiate appropriate countermeasures. It usually suffices to stop growth while the damage is repaired to prevent it from being passed on when cells divide. Only in extreme circumstances, the programmed death of the cell is triggered in order to eliminate irreparably damaged cells for the overall good of the organism. If these mechanisms are disturbed, or if they fail because of mutations, cancer may arise.

The p53 protein is a central component of the cellular response to stress. It belongs to the category of tumour suppressors whose deactivation is the first step on the way to cancer. Thus, in more than half of all human tumours, p53 has been deactivated by mutation. In the cell, p53 is regulated by a complex network of molecular interactions. The blueprint of this network was deciphered through many elegant molecular and cell biology studies (for an overview see: Kruse and Gu, 2009). This enables us to now investigate dynamic signal processing in the p53 network at the molecular level.

Time-resolved microscopy in living cells

Although all of our cells have the same genetic makeup, they often react differently to identical stress signals. On one hand, this is the consequence of fluctuations in molecular processes, which determine the precise conditions in individual cells at any given moment. On the other hand, cells are also influenced by their immediate environment, for example by the number of neighbouring cells.

Because of this heterogeneity, measurements of dynamic processes that capture a cell population as a whole render only a distorted picture of the actual behaviour of a single cell (Loewer and Lahav, 2011). In order to understand the dynamics of signal processing, it is essential to study the underlying processes at the level of individual cells. This calls for a technique that generates quantitative data with high temporal and spatial resolution. Time-resolved fluorescence microscopy can provide just that and enable the visualisation of molecular processes in living cells. The basic ingredients are fluorescent reporter proteins that can be fused with any gene product and then be transferred into cells. The intensity of these fluorescent reporters enables one to draw conclusions about the behaviour of the fusion partners studied. Various fluorescent proteins with defined colour spectrums are now available making it feasible to track multiple cellular processes in parallel. This in turn can provide information about both the interaction between different components of a particular signalling pathway and the interactions between signalling pathways themselves.

Alexander Loewer (Photo: David Ausserhofer/MDC).

In order to study signal processing within the p53 network in living cells, we produced fluorescent reporters for selected components of this signalling pathway. Combined with automatic microscopy, they allow tracking the cellular response to stress in real-time for several days. The imaging data generated is then computationally analysed and quantified. As it is hard to understand the dynamics of complex networks intuitively, we combine quantitative experimental data with mathematical models of the molecular interactions.

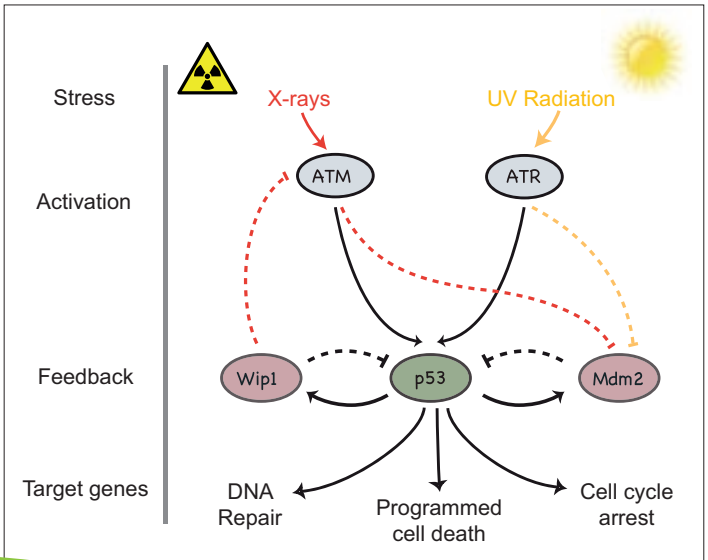
Digital and analogue signal processing in the p53 network

Using this approach, we investigated how information on cellular stress is encoded and processed in the p53 network. For example, when X-rays cause breaks in the DNA, special sensors are activated that stabilise the p53 protein. Normally, p53 is very short-lived. However, when stabilised during the stress response it accumulates in the nucleus and activates numerous target genes, which are involved in DNA repair, controlling cell division and inducing programmed cell death (Fig. 1). The particular set of genes activated by p53 will determine the cellular response to stress.

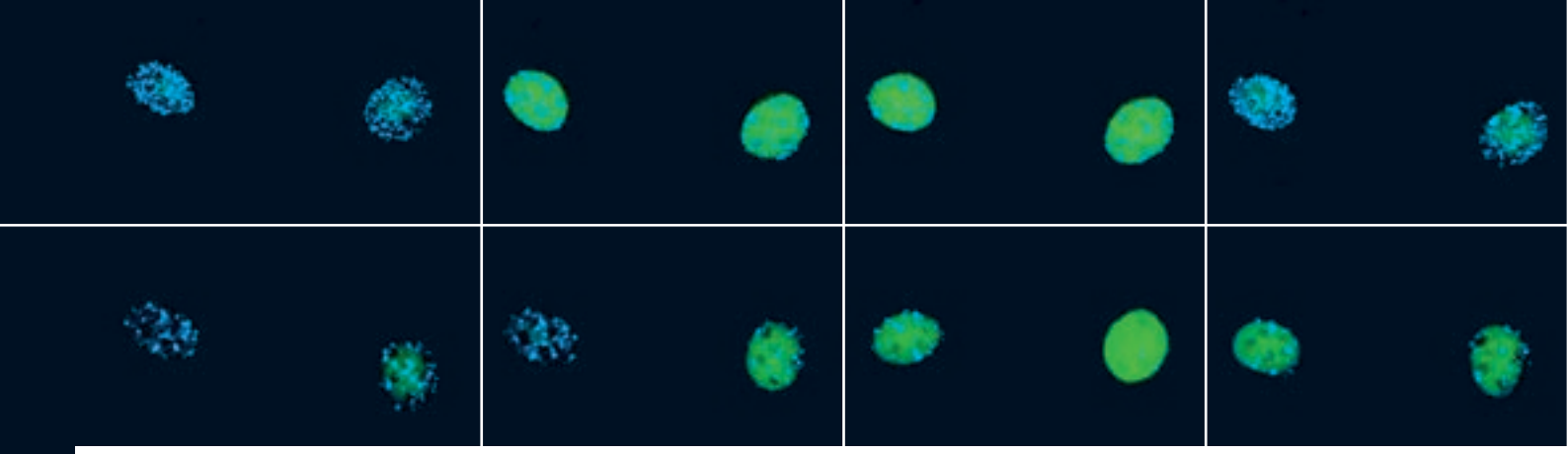
The target genes also include negative regulators of p53. These ensure that p53 is degraded again and that the response to stress is deactivated at the appropriate time. Such feedback loops are widespread regulatory mechanisms, not only in biology but also in engineering. They have a major impact on the dynamic behaviour of a system and can thus control its function. In the case of p53 they uniform pulses of p53 accumulation in response X-ray-induced breaks in DNA (Fig. 2). Surprisingly, neither the amplitude nor the duration of these pulses changes with the extent of the damage. Only the number of pulses provides information about the damage that has occurred. In this respect, p53 behaves as a digital system (Lahav *et al.*, 2004).

However, the network is flexible enough to encode information in more than one way. If a cell is hit by UV radiation, no DNA breaks in DNA, but defects in individual nucleotides. This damage also activates the p53 network, but it triggers pulses of

Figure 1: Diagram of the p53 signalling pathway



The tumour suppressor p53 is activated when a cell encounters stress, e.g. X-rays or UV radiation. The stress is detected by sensors that activate specific enzymes (ATM or ATR). These enzymes modify the p53 protein and stabilise it. p53 then activates its target genes that mediate the stress response. This involves arresting growth and repairing the damage, but also programmed cell death. Among the target genes are negative regulators of p53 (Mdm2 and Wip1) that ensure that p53 is deactivated again. Every stress activates specific network connections (red or orange arrows), which allows the signals to be encoded in different – digital or analogue – ways (Source: A. Loewer, MDC).



Time-resolved fluorescence microscopy enables visualising and quantifying dynamic processes in living cells. In this way, DNA damage and the cellular stress response can be studied in the same individual cell (Image: Alexander Loewer, MDC).

p53 accumulations with amplitudes and durations proportional to the degree of damage (Fig. 2). Therefore, p53 behaves like an analogue system in this case, where a stronger input signal leads to a stronger and more enduring output signal (Batchelor *et al.*, 2011).

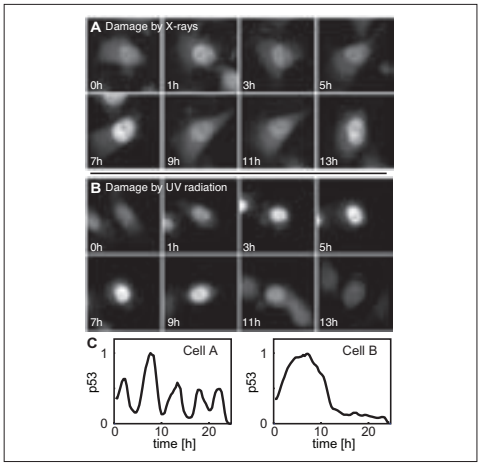
The difference between the two encoding modes can be ascribed to a few subtle changes in the network circuitry (Fig. 1). This allows the same molecular network to encode signals in various ways.

A molecular noise filter

While p53 has to react with high sensitivity to any damage that might lead to a mutation, it also needs a certain level of tolerance to naturally occurring damage in the cell, such as the damage caused by replication of the genome during cell growth. To understand how the p53 network differentiates between intrinsic damage of this kind and serious, externally induced damage, we observed cells during normal undisturbed growth. To our surprise, we repeatedly detected pulses of p53 even in apparently undamaged cells (Loewer *et al.*, 2010). These spontaneous p53 pulses have the same characteristics as those triggered by X-rays, but do not occur with the same regularity. More precise analyses showed that the pulses are not random, but occur during certain phases of the cell cycle. It is known that transient damage to the genetic material occurs during these phases. p53 reacts to this transient insults like an all-or-nothing signal and accumulates in a similar way as in response to severe damage. However, in contrast to lasting damage to the genetic material by external stressors, the transient damage did not trigger a full stress response, which would limit growth or even induce programmed cell death. In order to understand how spontaneous and regular p53 pulses differ at the molecular level, we used reporter cells to track the dynamics of p53 and the activity of a target gene in parallel (Fig. 3). As expected, p53 pulses after irradiation of the cells led to activation of the target gene, while similar pulses had no effect during normal cell growth. We were able to show that this difference is achieved by means of a filter in the p53 network (Loewer *et al.*, 2010).

This filter consists of certain modifications of p53 that are necessary for the transcriptional activation of target genes. While accumulation of p53 protein is triggered by the slightest stimulus, these activating modifications require constant input from the stress sensors. The combination of these two mechanisms, switch-like stimulation and subsequent filtering, enables the p53 network to react very precisely to externally induced damage to the genetic material and to distinguish it from the constant background noises caused by physiological processes.

Figure 2: The p53 response in living cells



Human breast cancer cells were stressed by different radiation, which produced either double-strand breaks in DNA (A) or damage to individual bases (B). The cells express a fluorescent reporter for p53, which enables following the dynamics of this protein in real-time under the microscope. Automated image analysis allows tracking individual cells and quantifying the intensity of fluorescence (C). Double-strand breaks in DNA trigger uniform pulses of p53 accumulation (digital encoding), while the strength and duration of the p53 response after damage to individual bases increases depending on the severity of the damage (analogue encoding) (Sources: Loewer *et al.*, 2010 and Batchelor *et al.*, 2011).

Complex signal processing

The example of the p53 signalling pathway shows the complexity of information processing in living cells. p53 is a dynamic signal integrator that can switch between digital and analogue encoding, depending on the stimulus. The signalling pathway reacts with high sensitivity to the slightest damage to the genetic material. Downstream filtering mechanisms then enable it to distinguish between spurious signals and lasting damage. In the future it will be important to clarify how the p53 signalling pathway is embedded in the cell's communication network and how it interacts with other signalling pathways. Only then we will be able to understand how the numerous external and internal signals are processed and control the cell's behaviour.

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The research project in brief:

The work described here was carried out at Harvard Medical School, Boston, MA, in the Department of Systems Biology. Since May 2011, the Signalling Dynamics in Single Cells research group headed by Dr. Loewer is continuing the work at the Berlin Institute for Medical Systems Biology (BIMSB) of the Max Delbrück Center for Molecular Medicine in Berlin-Buch. The BIMSB is funded by the German Federal Ministry of Education and Research (BMBF) and the Berlin Senate.

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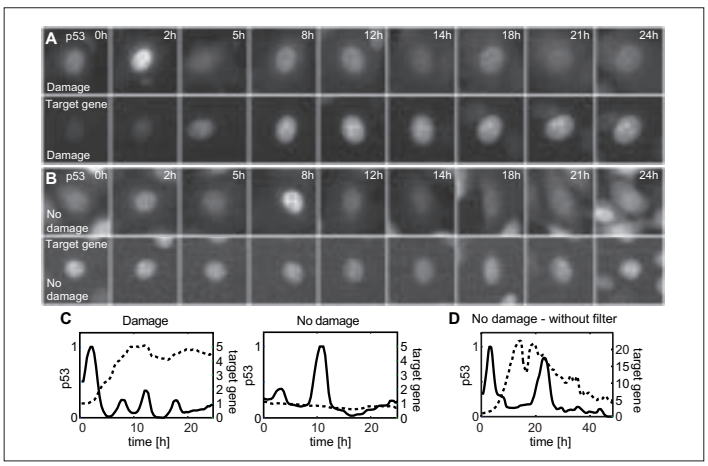
Figure 3: Signal processing in the p53 network



The signalling network uses a filter based on p53 modifications to distinguish between transient damage that occurs as background noise due to physiological processes and serious damage due to external influences. A dual reporter cell line was used to show that only regular p53 pulses following damage by radiation lead to the activation of a target gene (A). Similar pulses during normal cell growth do not trigger increased production of the target gene (B). This is illustrated by the quantification of the fluorescent signals (C). If the filter is selectively switched off by means of RNA interference, p53 pulses also activate target genes in normally growing cells (D) (Source: Loewer *et al.*, 2010).

evolutionary adaptation to living in the shade

A combination of experiments and modelling shows how plants can detect far-red light

by Julia Rausenberger, Eberhard Schäfer, Jens Timmer, Andreas Hiltbrunner und Christian Fleck

In order to detect light, humans and animals have light-sensitive proteins in the sensory cells of the retina. Analogously, plants too have light-sensitive proteins known as photoreceptors for detecting changes in their light environment. Phytochromes are photoreceptors that are activated by red light and are therefore optimally able to detect the red part of light. But plants also use phytochromes to detect far-red light, although their photophysical properties make them ill-suited to do so. By combining experimental approaches with mathematical modelling we found an explanation for this paradox of which scientists have long been aware (Rausenberger *et al.*, 2011).

Photoreceptors help in selecting a suitable development strategy

Light influences the life cycle of a plant in a variety of ways. Via the process of photosynthesis, plants derive from light the energy they need for survival. Unlike animals, which in adverse circumstances can simply move away and look for a better place, plants are stationary and have to adapt to the prevailing conditions in the place where they germinate. Various aspects of the light environment, such as the day length, the direction from which the light comes or its spectral composition give plants important information about their environment. As days grow shorter, for example, plants start to prepare for approaching winter, or a change in the light spectrum enables them to recognise rivals before they become a threat to survival.

To detect light, plants use various light-sensitive proteins known as photoreceptors. These phytochromes, cryptochromes and phototropins give plants the ability to record important parameters of their environment. In order to absorb light, all three photoreceptor families depend on chromophores.

Phytochromes contain phytochromobilin, a linear tetrapyrrole, as a chromophore (Fig. 1a), while cryptochromes and phototropins have flavin-based chromophores (Fig. 1b). Already very young seedlings choose between two development strategies, depending on the light (Fig. 1c). After germinating, in the absence of light they use their limited stocks of storage substances for more elongated growth in order to reach the light, thereby enabling photosynthesis and thus photoautotrophic growth for the little plant (Greek *photos* = light, *autotroph* = self-feeding). This strategy is called skotomorphogenesis. As soon as light is available, the plant switches to a second development strategy known as photomorphogenesis, where the main emphasis is on growing more leaves and optimising the photosynthesis process. Thus, seedlings with the same genetic background can develop very differently, depending on the environmental and light conditions. Over the course of the earth's history, as plant growth became increasingly dense, a further characteristic of plants became important – the ability to survive in the shadow of other plants. Beneath a dense cover of vegetation, the ratio of far-red light is strongly increased because the chlorophyll in the leaves of overshadowing plants filters out the blue and red light part of the sunlight. In these conditions, detecting far-red light is indispensable in order to enable the transition from skotomorphogenesis to photomorphogenesis after germination and therefore to photoautotrophic growth.

Phytochromes absorb most light in the red light region, but can achieve maximum effect in far-red light

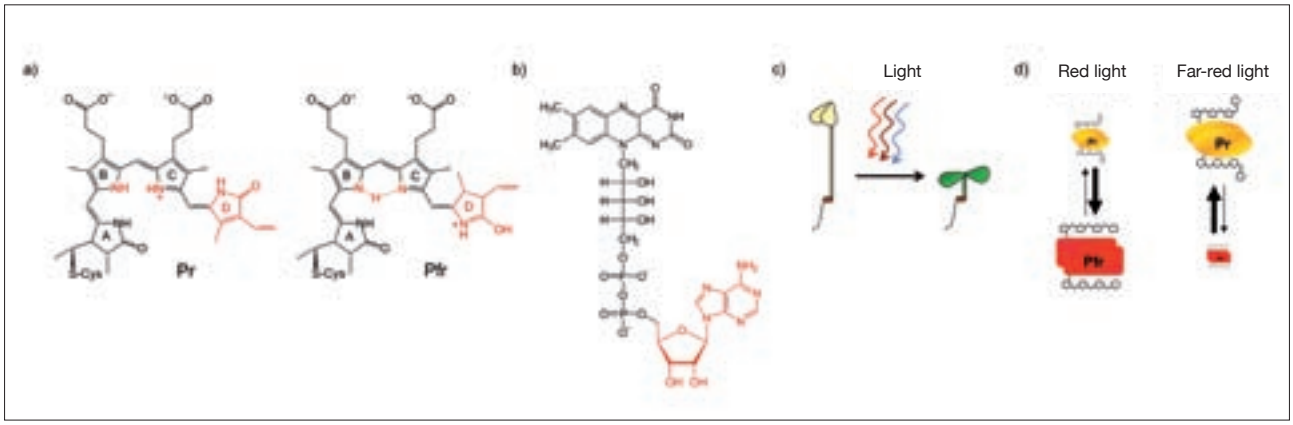
In the red and far-red regions of the light spectrum (625–740 nm), light quality and quantity is detected by the photoreversible phytochrome system. A phytochrome consists of a protein component and a light-absorbing component, the chromophore. This chromophore, phytochromobilin, and the phytochrome molecules exist in two spectroscopically differentiable forms, the Pr and the Pfr form (Figs. 1a and 1d). The Pfr form is

Researching light detection in plants: from left to right Christian Fleck, Florian Wüst, Eberhard Schäfer, Julia Rausenberger, Andreas Hiltbrunner and Jens Timmer (Photo: Christian Fleck).

considered to be the physiologically active form, while the Pr form is inactive. By absorbing light, the two forms can be converted into each other. While the rate of conversion from the Pr to the Pfr form is greatest in red light, the rate of conversion from the Pfr to the Pr form is highest in far-red light (Fig. 1d). As a result, in red light 85% of phytochrome molecules are in the physiologically active Pfr form, while the proportion of Pfr in far-red light is only 3%. Correspondingly, one would expect phytochrome-dependent responses to be activated by red light and inactivated by far-red light, i.e. that phytochromes would act as "light switches" switched on by red light and switched off by far-red light.

Plants have various types of phytochromes that differ hardly at all in terms of photophysical characteristics. The two most important phytochromes are phytochrome A and phytochrome B. Phytochrome B behaves as one would expect a phytochrome to do: It has the strongest effect in red light, where the Pfr proportion is highest. In contrast, the effect of phytochrome A has been found to be greatest in far-red light. Phytochrome A is the photoreceptor that enables plants to survive beneath a dense cover of vegetation by stimulating the transition to photoautotrophic growth in far-red light. The paradox that the effect of phytochrome A is greatest in far-red light despite the fact that in that part of the spectrum only 3% of all phytochrome A is in

Figure 1:



a) Structure of phytochromobilin, a linear tetrapyrrole, in the Pr and Pfr form (differences in red). In phytochromes, phytochromobilin is covalently bonded to a cysteine (Cys) (Source: http://de.wikipedia.org/w/index.php?title=Phytochrom&oldid=92061197).

b) Cryptochromes and phototropins, both blue-light receptors in plants, unlike phytochromes, have a flavin-based chromophore. Cryptochromes have a FAD (overall structure), while phototropins have a FMN (black) (Source: http://de.wikipedia.org/w/index.php?title=Flavin-Adenin-Dinukleotid&oldid=92551360).

c) Skotomorphogenesis vs. photomorphogenesis: Seedlings that grew in the dark (left) use their reserves to grow more elongated, whereas seedlings that grew in the light (right) strive to optimise photosynthesis by growing more leaves (Source: J. Rausenberger, A. Hiltbrunner).

d) The photoreversible phytochrome system: Under the influence of red light the Pr form is converted into the physiologically active Pfr form, which reverts through far-red light back to the Pr form. When exposed to red light, around 85% of phytochromes are in the Pfr form, as opposed to only around 3% in far-red light (Source: J. Rausenberger, A. Hiltbrunner).

Figure 2: *Arabidopsis thaliana* seedlings that have grown for four days in far-red light. The seedlings in the middle have lower and those on the far right higher quantities of the protein FHY1 than is present in wild type seedlings (left) (Photo: A. Hiltbrunner).

the active Pfr form, has preoccupied plant researchers for more than 50 years. In all that time, no convincing explanation of this phenomenon, otherwise known as the high-irradiance response (HIR), was found.

Initial mathematical analyses of this problem led Schäfer in 1975 to a cyclical pattern of reaction as an interpretation of what was known at the time about the kinetics of the phytochrome. Although this model was an important step towards understanding HIR, the actual mechanism at the cellular and molecular level was still not understood.

Further research in the model plant *Arabidopsis thaliana* fundamentally changed the simple picture of the phytochrome as a "light switch". This started with the discovery that in the dark phytochromes are localised in the cell cytosol and are transported to the nucleus only after activation by light, i.e. after conversion into the active Pfr form (Kircher *et al.*, 1999, 2002; Yamaguchi *et al.*, 1999). This was followed by the discovery that two helper proteins, FHY1 and FHL, are needed to transport phytochrome A to the nucleus (Hiltbrunner *et al.*, 2006; Rösler *et al.*, 2007; Genoud *et al.*, 2008). The FHY1 and FHL proteins interact specifically with the Pfr form of phytochrome A, but detach themselves from it after it converts into the Pr form. Surprisingly, the quantity of FHY1 and FHL is much lower than the quantity of phytochrome A they transport into the nucleus. It was therefore postulated that once their work is done, the proteins are transported back out of the nucleus into the cytosol so as to be available for multiple transport cycles. Plants that contain higher quantities of FHY1 show greater inhibition of elongated growth in far-red light and less when the quantity of FHY1 is reduced (Fig. 2). This finding supports the assumption that the quantity of FHY1/FHL has a limiting effect on nuclear transport and on the action of phytochrome A.

One problem of many previous approaches to solving HIR was that they tried to explain the phytochrome system using a simple "light switch" model and did not take the actual dynamics of the photoreceptor and its nuclear transport and interaction with other proteins into account. A successful theoretical approach had to take account of both the light-dependent nuclear transport and the particular dynamics of FHY1 and FHL, in addition to the specific phytochrome dynamics. Precisely this approach provided the possibility of decoding the unsolved problem of HIR and of being able to explain it at the molecular level (Fig. 3a).

Close combination of experiment and theory decodes counterintuitive high-irradiance response

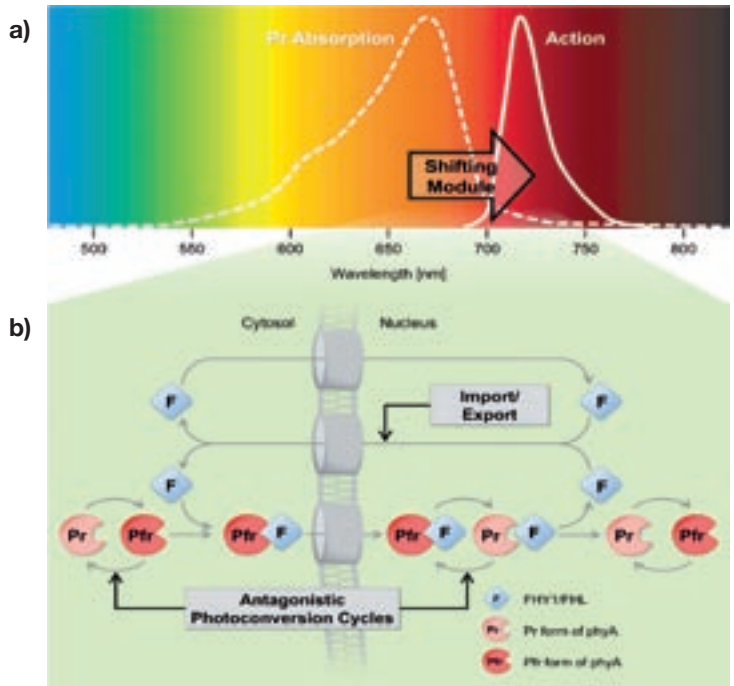
In laboratory experiments with transgenic *Arabidopsis* plants we were able to show that the helper proteins FHY1 and FHL needed for the nuclear transport of phytochrome A detach themselves from it in the nucleus and migrate back into the cytosol, where they are available for further transports. We were also able to identify a mutated form of phytochrome A that was constitutively present in the physiologically active Pfr form and therefore binds to FHY1 and FHL permanently. Surprisingly, the effect of this mutated form was not greater than the wild type, but rather noticeably reduced. Further experiments showed that nuclear transport of the mutated form was less efficient than of the wild type. Based on the findings of these experiments, we developed a mathematical reaction model for the effect of phytochrome A. The goal was to find out whether this model reflected HIR and what reactions in this network were fundamental to phytochrome A's effectiveness in far-red light.

Due to the high number of open parameters that could not be defined experimentally, we chose a qualitative approach that could be guided by the following question: Are there combinations of parameters for which the reaction model set up (Fig. 3b) meets all the conditions that were previously defined on the basis of experimental observations? Systematic testing of 1,000,000 parameter combinations resulted in approximately 6,000 combinations that met all the predefined conditions. Although maximum effect in far-red light was not one of the criteria for choosing the 6,000 parameter combinations, nearly

all these combinations resulted in a maximum effect in far-red light – and not in the red region of the light spectrum as one would expect, given the photophysical characteristics of phytochromes.

Maximum effect in far-red light, the crucial characteristic of HIR, is therefore an intrinsic characteristic of the reaction model shown in Fig. 3b. However, these computer simulations did not mean that HIR had been understood. Although the mathematical reaction network showed the desired characteristics, it was unclear which were the key components, and how they must be interlinked in order to push the maximum effect from the red light into the far-red light. To find this out, we switched to an abstract way of looking at the problem that was inspired by synthetic biology and designed theoretical networks with phytochromes. If we were to start with the smallest possible network and augment it by systematically adding more and more components, at some point we must find the simplest network that showed the necessary maximum effect in far-red light. It must then be possible to find this smallest network, which we called the HIR module, again as a sub-unit of the phytochrome A reaction network in Fig. 3b. Interestingly, a linear network with just three phases showed the required maximum effect of HIR at a wavelength of 720 nm, i.e. in far-red light. Mathematical analysis of this network yielded a surprisingly simple insight: Two photoconversion cycles operating in opposite directions (i.e. Pr→Pfr and Pfr→Pr), combined with a system in which continuous synthesis and breakdown takes place, which is therefore not in balance, are the long sought-after essential network elements. Furthermore, these key components could also be identified as a structural element in the more extensive phytochrome A reaction network shown in Fig. 3b. In laboratory experiments we were also able to establish that in the plant the proteins responsible for transporting phytochrome A to the nucleus, FHY1 and FHL, link the two opposite phytochrome A photoconversion cycles with each other (Fig. 3b).

Figure 3:



a) A previously unexplained phenomenon: How can a photoreceptor, whose maximum absorption is in the red light region, achieve a maximum effect in far-red light?

b) Main components of the HIR module: Two photoconversion cycles operating in opposite directions and nuclear transport

(Source: A. Hiltbrunner, modified after Rausenberger *et al.*, Cell 2011).

Model calculations show that several concatenated HIR modules lead to a narrowing of the phytochrome A spectrum of activity and that with four HIR modules one obtains a spectrum of activity that best accords with an experimentally defined spectrum of activity for phytochrome A. From an ecological viewpoint, narrowing the spectrum of activity makes good sense, since it allows the plant to separate responses to far-red light much more precisely from those to red light that are mediated by phytochrome B. Laboratory experiments confirm that several HIR modules have to be present in the plant. In future studies we aim to identify by means of experiments the HIR modules predicted by model calculation.

Earlier attempts to explain HIR reached the conclusion that neither the Pr nor the Pfr form was responsible for phytochrome A's detection of far-red light, but an "unknown, intermediary form". However, our approach shows that the Pfr form is indeed sufficient for signal transduction, provided that two conversion cycles operate beforehand in opposite directions. This approach delivers a mechanical explanation of how HIR, and therefore the detection of far-red light, can function at the molecular and cellular level. Therefore, in the course of evolution, plants have not developed a completely new photoreceptor for detecting far-red light. Rather, they use a photoreceptor that is actually optimal for detecting red light and integrate it into a network. This network as a whole achieves a maximum effect in far-red light and thus enables plants to survive beneath a dense cover of vegetation.

The research project in brief:

Title: Light Perception of Plants

Funding: DFG (SFB592, GRK1305, EXC294); BMBF - Freiburg Initiative in Systems Biology 0313921 (FRISYS)

Participants: Julia Rausenberger, Anke Tscheuschler, Wiebke Nordmeier, Florian Wüst, Jens Timmer, Eberhard Schäfer, Christian Fleck, Andreas Hiltbrunner

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An interview with Professor Dr. Ulrike Gaul

Gene Center of the University of Munich (LMU)

For over two decades, the internationally renowned scientist Ulrike Gaul has studied how genes control the development of the fertilized egg to a full-fledged organism. She is aided by *Drosophila melanogaster,* the famous laboratory fly, in which developmental processes can be investigated in a paradigmatic fashion. At the Gene Center of the Ludwig-Maximilians-Universität Munich, the Alexander von Humboldt professor uses systems biological approaches to understand how the countless genes and proteins collaborate in the choreography of life.

Professor Gaul, when you returned to Germany from the Rockefeller University in New York two years ago you brought your flies back with you. What is so important about these flies?
They are all genetically modified flies, mutants that lack certain genes, or transgenic flies into which special genes have been inserted. We generated many of them ourselves, which required a great deal of work, and that is why we took them with us. They are not just any old flies, but genetic rarities that help us do our work.

What work is that?
Basically, we would like to understand how a complex organism with all its different cell types, tissues and organs arises from a single cell – the fertilised egg. For this to happen, the egg must divide in a controlled manner, the daughter cells must specialise in different tasks, and the entire body plan of the animal must be generated. As a developmental biologist I would like to know how this body plan is established in consecutive steps – this is really the fundamental question all developmental biologists are interested in. In addition, I approach it from a decidedly systems-biological perspective.

Two years ago you set out to strengthen systems biology – currently one of the most dynamic areas of biological research – in Germany. How is your research going?
Part of our work is devoted to the early development of the *Drosophila* embryo, and especially the so-called segmentation process, during which the developing fly is partitioned into different body segments. This is actually an old topic – the genes that are involved in this process were discovered by Christiane Nüsslein-Volhard, Eric Wieschaus and colleagues in the early 1980s. But what we now wish to understand is how these approximately 100 genes collaborate and interact. It has become clear in the meantime that nearly all these genes encode transcription factors, or proteins that bind to the DNA in order to switch genes on or off. These transcription factors form a complex network, in which groups of genes are activated sequentially through combinatorial interaction. The result is an increasingly fine subdivision of the embryo into different regions. We want to know how this regulatory network functions – you might also say we want to decipher the underlying regulatory code.

What do you mean by 'regulatory code'?
The genetic code has been known for a long time. Certain sections or sequences of DNA – the genes – contain the blueprint for proteins, and we know exactly which DNA sequences stand for which protein sections. However, we know much less about the regions of DNA that do not code for proteins. These regions, which, incidentally, are much longer than the genes themselves in all higher organisms, contain binding sites for transcription factors and thereby control when genes are read off and activated. Little is currently understood about how these regulatory sequences work and how the instructions are encrypted in the DNA. We would love to crack that code.

Ulrike Gaul holds the Chair of Organismic Biochemistry at the University of Munich's Department of Biochemistry (Photo: private).

And where does systems biology come in?

The challenge is to gain a holistic view: How do all these complex interactions between transcription factors and regulatory DNA work together to create distinct patterns of gene activity in the embryo? To answer this question we collated all existing research findings and then asked whether these data are sufficient to devise mathematical models that recapitulate the pattern formation process.

Can you be more specific?

Our concrete question was whether our current knowledge about the participating transcription factors, their distribution in the embryo, and their affinity to bind to certain DNA sequences is sufficient to develop a model for segmentation that captures reality. We published this work in *Nature* in 2008. It was the first attempt to predict gene activity in this network from the binding of transcription factors to DNA and thus from the DNA sequence. Pattern formation in the early *Drosophila* embryo had been modelled before, using so-called reaction diffusion models. But such models disregarded the mechanistic molecular core of this process – the binding of transcription factors to the DNA. We put it at the center of our approach. This is systems biology of a gene regulatory network.

What is the aim of such research?

The aim of this kind of modelling is always to predict the reaction of the system to intervention and, ultimately, to influence biological processes in a targeted fashion. Frequently we know the identity of the individual players in a process. Systems biology means asking how the players interact to jointly achieve a certain result and how this activity is coordinated. And for that we need our flies. What happens, for example, to a mutant in which a gene is either missing or overexpressed? Can I predict with certainty what will happen under these altered conditions? Only if I can forecast a system's behaviour will I have fully understood it, and only then can I really manipulate it in a precise manner.

Are we currently able to make such precise predictions?

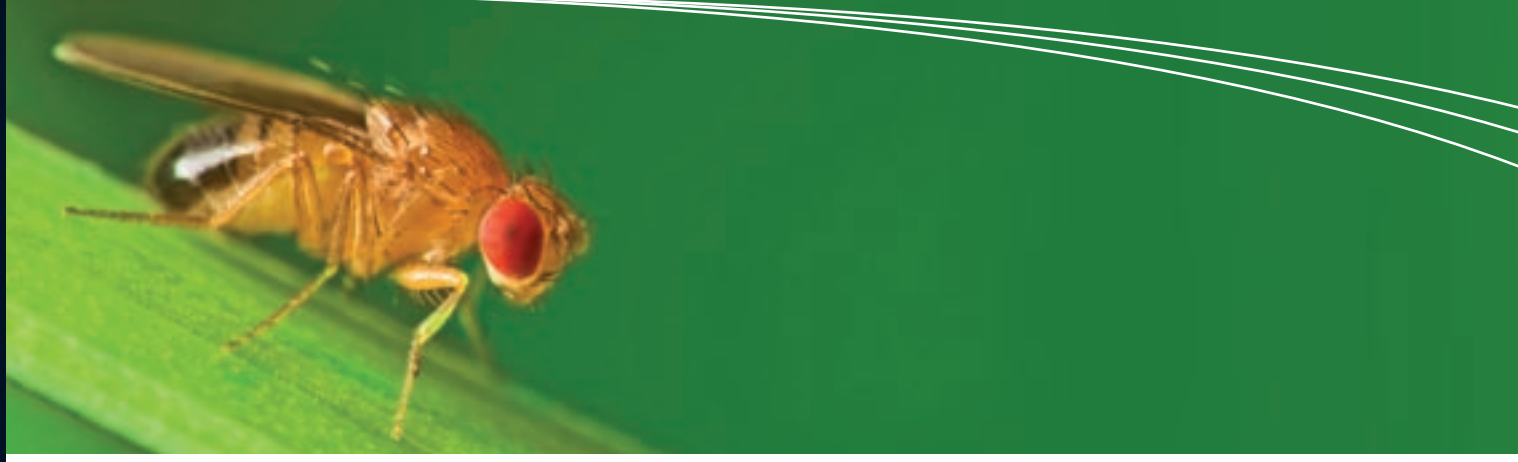
No, certainly not. When we created our model, we also gained insight into what we don't know. For example, we are missing information about the ubiquitous transcription factors, which are present uniformly in all embryo cells. Using genetic and bioinformatics methods we have begun to search for gene activators and other missing factors. We also don't understand well enough how the binding strength of transcription factors depends on the DNA sequence. We are now carrying out targeted experiments in order to tackle these issues with a higher quantitative resolution.

Back to reductionism, then?

I am not sure I would call it reductionism. I would say it is the hermeneutic circle. You find out what causes the error and then try to take better measurements to improve the situation. Guided by the errors or the problems in modelling, one can decide which experiments to prioritize. Generally speaking, systems biology still lacks hard quantitative data. That is why one of our most important goals is to generate excellent quantitative data for the biological system we are investigating.

Which experiments are particularly important to complete the hermeneutic circle?

Aside from measuring the binding strength of transcription factors to the DNA, we are mainly interested in the regulatory grammar, in other words in discovering how the different binding sites must be arranged within the DNA in order to jointly control the activity of a gene. We are investigating this on the one hand through evolutionary comparisons with



Drosophila melanogaster (Photo: © Studiotouch – fotolia.com).

related *Drosophila* species, and on the other hand by constructing synthetic DNA sequences that we then insert into the genome of *Drosophila*.

Is systems biology the key to achieving further progress in developmental biology?

Yes, I believe so. If one wants to understand the development of an organism, there is ultimately no way around a systems biological approach. That does not only apply to developmental biology, by the way. Any biological process you can image consists of many different components that are jointly responsible for a certain outcome. Whether it is stem cells or understanding cancer, we always need to know which players are involved and how they interact with one another. Only then can we hope to influence processes and, for example, fight cancer.

What is needed to reach this goal?

One essential prerequisite is exact measurements – and the experimental methods to make such measurements possible. In biology, data have typically been qualitative or at best semi-quantitative, but in the end one needs numbers, not just a yes or no. Systems biology seeks to transcend simple binary models and to do justice to the quantitative character of all life.

If you think ahead five years, what would you like to have accomplished by then?

I would like to have a more precise understanding of regulation in gene networks and of their behaviour under different conditions. Segmentation serves us as a kind of testing ground where we can try out many things, because it is a well-established system, where a lot of data and many experimental tools are available. However, we are also interested in much more complex systems, such as the role glial cells play in the development of the nervous system, or the fly's imaginal discs, from which wings, sex organs, legs, eyes and antennas develop within a few days during metamorphosis.

Schematic depiction showing the gradual refinement of expression patterns within the segmentation gene network of *Drosophila*

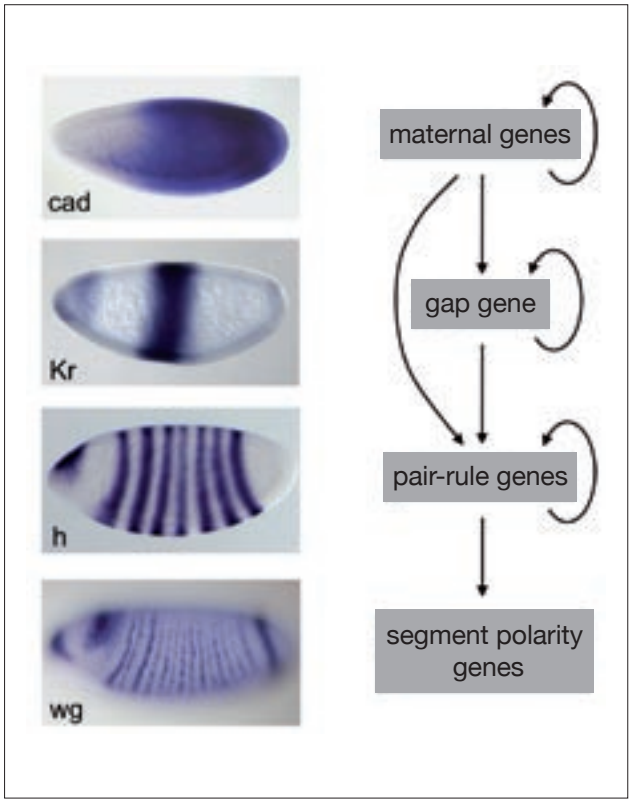


Chart: modified from Schroeder *et al.*, PLoS Biology 2004.

You have had a storybook career. What would your advice be to young people at the beginning of their scientific careers? Is there a specific strategy they should follow?

You need to be genuinely enthusiastic about science, and you must have a burning question that you really want to answer. Otherwise you will not survive in the tough world of scientific research. Science demands complete commitment. But one also needs a certain playfulness and the courage to question things. You need constant intellectual creativity. A successful career in science cannot be planned – if you seek a secure, linear career, go elsewhere. In my view it is also very important to have worked abroad for some time, both for your personal and for your scientific development. Goethe spent years abroad – and in his day good craftsmen also knew that you begin as an apprentice, then set out as a journeyman before, hopefully, becoming a master of your profession.

Interview by Claudia Eberhard-Metzger.

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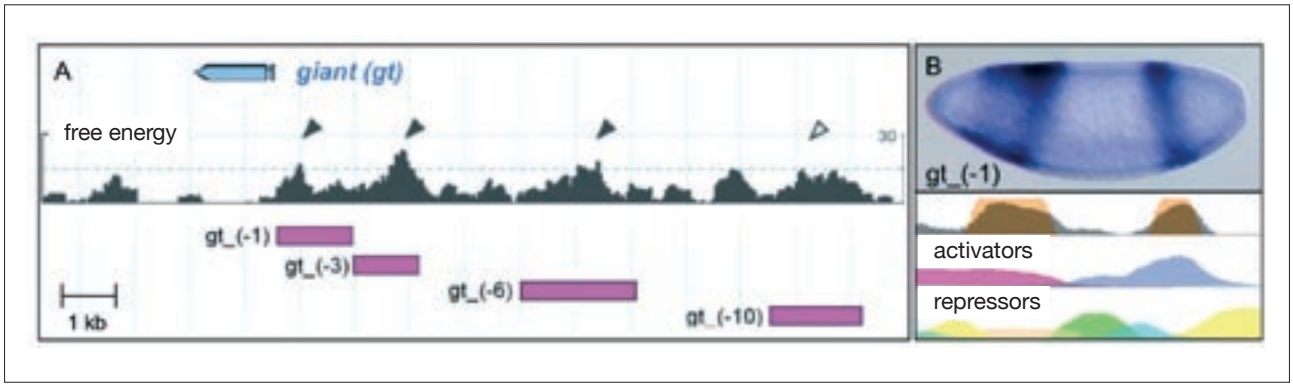
Systems biological investigation of the segmentation gene network



Systems biological investigation of the segmentation gene network. Detection of regulatory DNA regions by computational search for transcription factor binding sites (A) and prediction of the resulting gene activity for one of the regions through thermodynamic modelling of transcription factor–DNA interactions (B) (modified from Schroeder *et al.*, PLoS Biology 2004, and Segal *et al.*, Nature 2008).

ICGC – the international cancer genome consortium

Working towards personalised cancer therapies

by Ursula Weber, Reiner Siebert, Holger Sültmann und Daniela Wuttig

The goal of the International Cancer Genome Consortium (ICGC), founded in 2008, is to genetically catalogue the 50 most common tumour diseases. This involves using next-generation sequencing (NGS) to itemise 500 tumours and 500 controls for each type of tumour and analyse them by means of bioinformatics. The data is pooled in a large database and made available to the international research community.

There are currently three German ICGC participants: the PedBrainTumor project (paediatric brain tumours), the prostate cancer project (early-onset prostate cancer) and the ICGC MMML-Seq project (Molecular Mechanisms in Malignant Lymphomas).

Many cancers are still incurable. Even in cancers where cells cannot be differentiated under the microscope, some patients respond very well to a specific therapy while others do not respond at all. This indicates that the reasons for the onset and spread of cancers can be very diverse. They can often be traced back to the individual genetic make-up of the tumour.

Sequencing human genomes

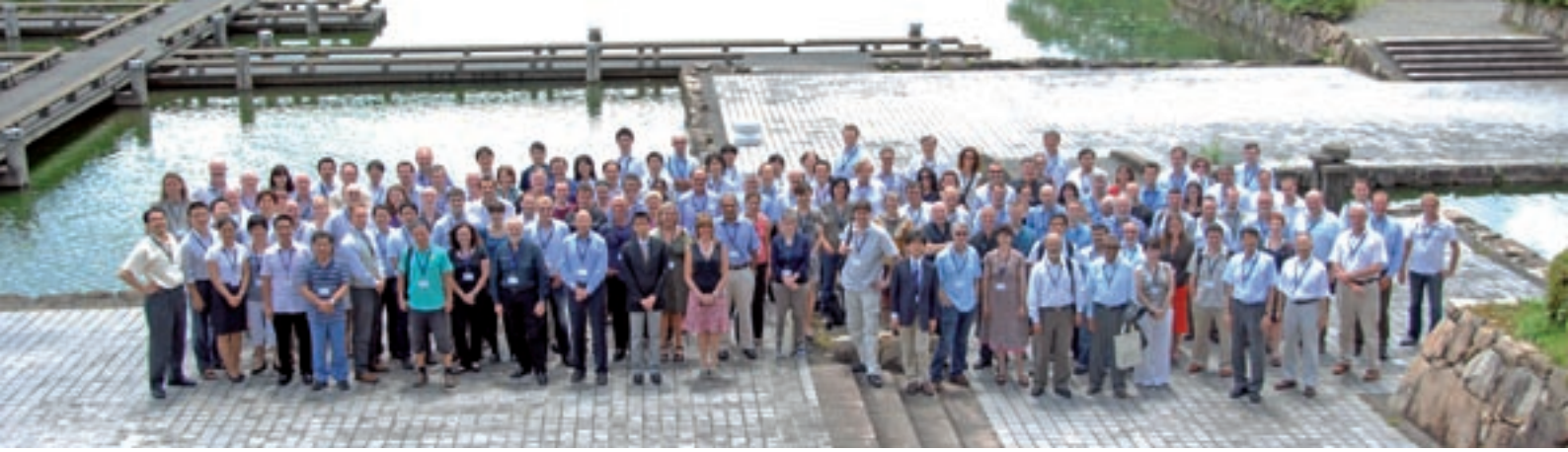
The base sequence of the first human genome was officially presented in 2003 after more than 10 years of sequencing. Since then, the development of sequencing technologies has advanced rapidly, so that sequencing a genome now takes only about a week and has become financially affordable (see also the article on page 28 of this issue). The International Cancer Genome Consortium (ICGC; www.icgc.org), established in 2008, coordinates the sequencing of genomes from different types of tumours worldwide. This enables studies to be carried out on the basis of uniform standards and avoids duplication so that resources around the world can be optimally deployed. The consortium's goal is to genetically characterise the 50 most common types of tumour. This is done using next-generation sequencing (NGS) technologies. The aim is to generate a comprehensive catalogue of DNA mutations and gene activities (transcription, methylation) as well as epigenetic changes in the most frequently occurring tumours. In order to make statistically reliable statements about the frequency of mutations in the genetic code, at least 500 patient genomes should be sequenced for each tumour entity. In addition, as a reference for each tumour entity the same number of genomes from healthy cells of the tumour patients should be sequenced.

ICGC Data Coordination Center

One of the goals of the ICGC is to pool data with the international research community so as to improve and accelerate research into the causes and treatment of cancers. In doing so, data protection is respected and the study participants' right to privacy is protected. No connection may be made between a specific individual and the corresponding genetic data. The data is divided in two different categories: A public database and one with controlled access which is made available to scientists upon request.

Benefit to patients

Along with developing new therapies, it will be essential to subdivide a tumour entity into different biological or genetic subtypes insofar as this is of prognostic significance and different therapies can be derived from doing so. This is already possible in some types of cancer with the help of different markers. By dividing tumours into different subtypes, some patients can be spared chemotherapy with severe side effects,



ICGC cancer researchers at the 5th ICGC workshop in Kyoto, Japan, July 2011 (Photo: icgc.org).

while other groups may only respond to treatment with especially intensive therapies. In addition, from genome information it may be possible to derive effective alternative therapy approaches that enable direct intervention in accordance with the molecular changes found. This is a further major step towards personalised medicine.

Podcast:
www.swr.de/swr2/programm/sendungen/wissen/archiv/erbgut/-/id=660334/nid=660334/did=8557084/11bpka/index.html
Internet:
www.icgc.org

References:

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German ICGC projects

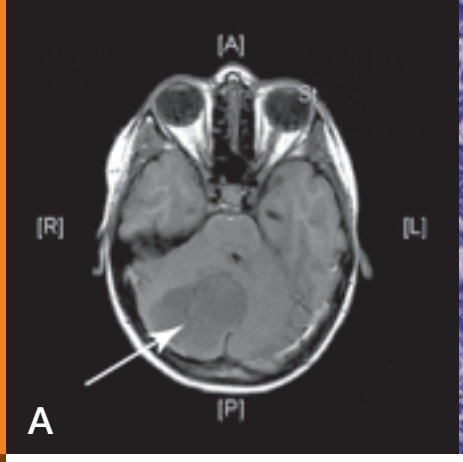
I. ICGC PedBrainTumor – paediatric brain tumours

The two most common paediatric brain tumours are medulloblastoma and pilocytic astrocytoma (Fig. 1), which account for around 300 cases per year in Germany. Treatment of these tumours places a great strain on the young patients. Moreover, even after successful treatment the children often suffer from the long-term effects of therapy. The goal of the PedBrainTumor project is therefore to contribute to the International Cancer Genome Consortium by developing new, gentle therapy methods for children suffering from these brain tumours. At the same time, it aims to identify those patients who will really benefit from more intensive therapy.

The PedBrainTumor project was launched at the start of 2010 within the framework of the International Cancer Genome Consortium and is receiving funding for a period of five years from German Cancer Aid (DKH) and the German Federal Ministry of Education and Research (BMBF). During this time, the plan is to sequence a total of 600 tumour samples of paediatric medulloblastomas and pilocytic astrocytomas as well as the same number of control samples and to analyse them by means of bioinformatics. Internationally renowned scientists from different institutes and universities are participating in this project. Only by combining their diverse expertise, it is possible to carry out this project successfully (Fig. 2).

Although this concerns highly aggressive tumours, initial findings indicate that medulloblastomas in children have significantly fewer mutations than all adult tumours examined so far. Moreover, there appears to be a direct correlation between the number of tumour-specific mutations and the patient's age. The significantly lower number of tumour-specific mutations per tumour will presumably make it easier to identify the causal mutation(s) in each tumour. This could then make it easier to select targeted medicines.

The extreme genetic heterogeneity of this type of tumour and the spectrum of mutated genes have proved to be major challenges. Presumably, many genes in which no cancer-specific mutations had previously been found have mutated in the medulloblastoma.



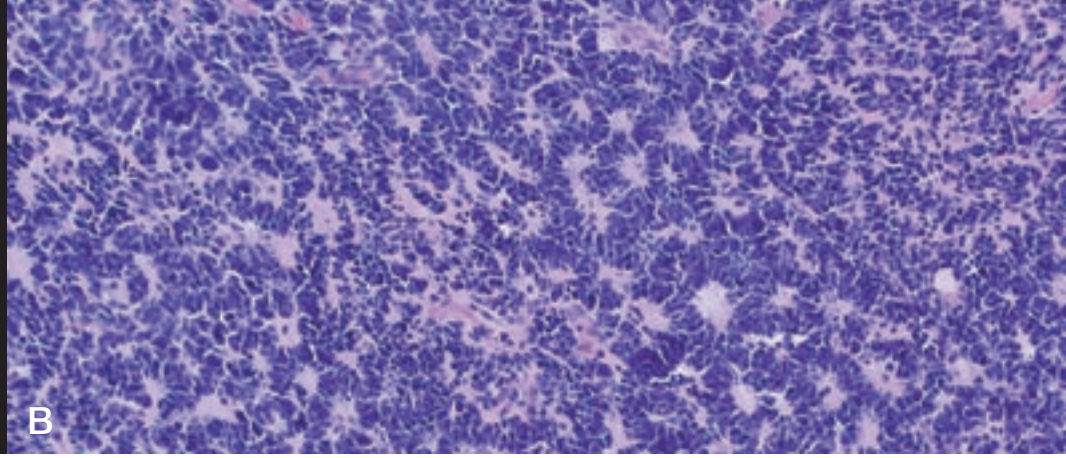


Figure 1: MRI and histological tissue section of a medulloblastoma (A and B) and a pilocytic astrocytoma (C and D) (Source: Heidelberg University Children's Hospital).

The research project in brief:

Project name:

PedBrainTumor is the first German contribution to the International Cancer Genome Consortium and is funded by both German Cancer Aid (DKH), which is responsible for the first funding period, and by the German Federal Ministry of Education and Research (BMBF) with a total budget of €15 million over five years.

For further information, please visit:

www.pedbraintumor.org

Participating partners:

Coordination: Peter Lichter, Roland Eils (deputy coordinator), Ursula Weber (project management), German Cancer Research Center (DKFZ) Heidelberg.

Tissue bank: Andrey Korshunov, Olaf Witt, Stefan Pfister, DKFZ and Heidelberg University Hospital.

Genotyping: Michael Taylor, Paul Northcott, SickKids Hospital, Toronto, Kanada.

Reference pathology and quality control: Guido Reifenberger, Düsseldorf University Hospital.

Isolation of analytes (DNA, RNA): Christof von Kalle, Manfred Schmidt, National Center for Tumor Diseases (NCT) Heidelberg.

Genomic DNA sequencing: Stefan Pfister, Peter Lichter, DKFZ Heidelberg.

Paired end mapping: Jan Korbel, The European Molecular Biology Laboratory (EMBL) Heidelberg.

Methylome analysis: Bernhard Radlwimmer, DKFZ Heidelberg.

Transcriptome analysis: Marie-Laure Yaspo, Hans Lehrach, Max Planck Institute for Molecular Genetics, Berlin.

miRNA analysis: Pablo Landgraf, Arndt Borkhardt, Guido Reifenberger, Düsseldorf University Hospital.

Bioinformatics: Benedikt Brors, Marc Zapatka, Roland Eils, DKFZ and Heidelberg University.

Data Management: Roland Eils, Chris Lawerenz, Jürgen Eils, Heidelberg University and DKFZ.

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Figure 2: Workflow: classification, sequencing and analysis of a tumour sample

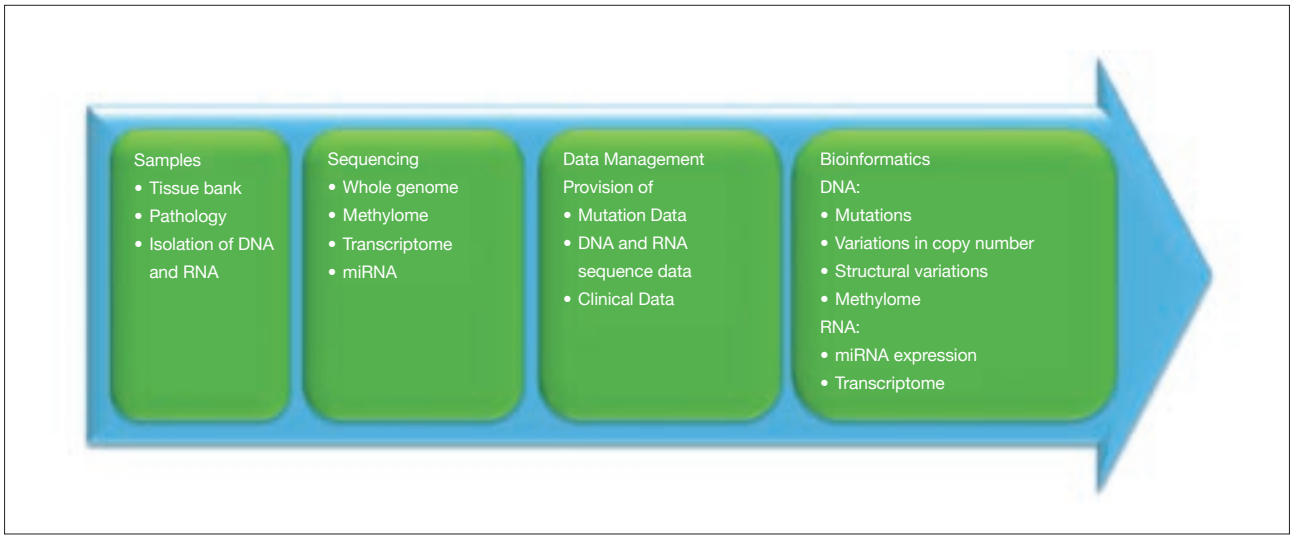
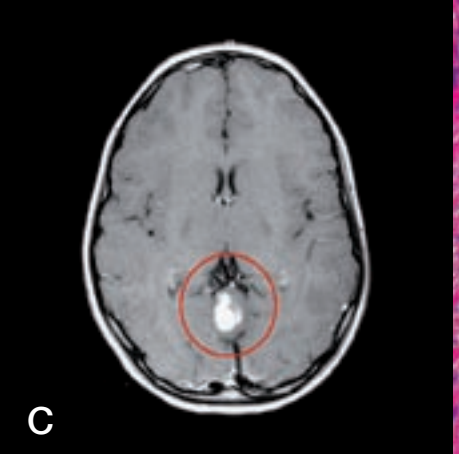
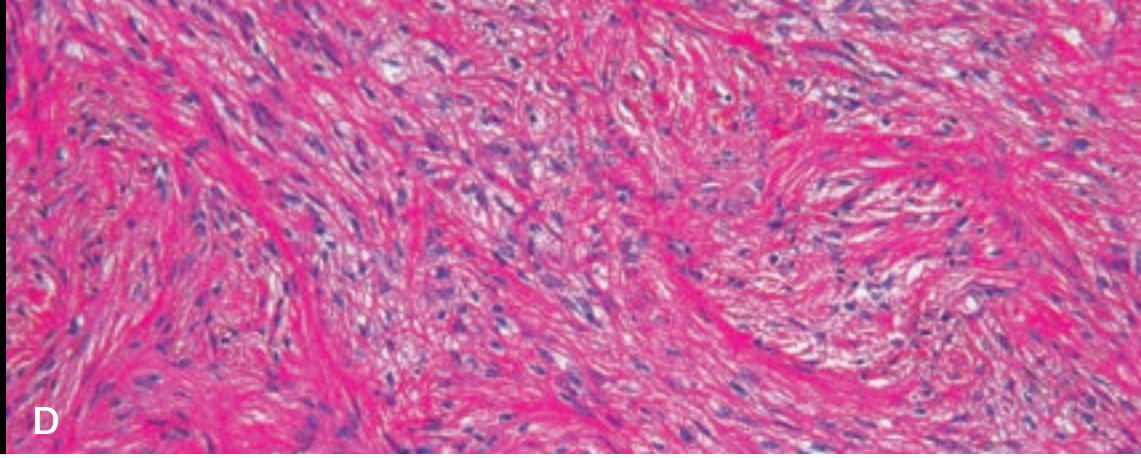


Chart: Ursula Weber





II. The prostate cancer project

Prostate cancer is the most common malignant tumour in men. Currently, more than 60,000 cases are diagnosed each year in Germany alone. As our population continues to age, its incidence is expected to double in the next 20 years. Prostate cancer usually occurs in men between the ages of 60 and 70, although around 2% of men are under 50 years old when prostate cancer is diagnosed. The second German ICGC study "The genomes of early onset prostate cancer" has been focusing on these early-onset cancers since November 2010.

In understanding the biology of prostate cancer, tumours in younger patients could play a key role for the following reasons:

- They may bear a disproportionately large number of tumour-associated **(driver)** mutations that are causal in the emergence and progression of tumours.
- They manifest changes that probably represent an early stage of conventional prostate cancer and could be useful in the **early diagnosis** of conventional prostate cancers.
- The molecular changes in them could contribute towards the development of new, **targeted therapeutic agents**. Targeted treatment of tumours is essential in this group of young patients.
- Early-onset prostate cancers may in part be hereditary. A comparison with prostate cancers in the typical age structure may provide indications of the **involvement of hereditary prostate cancers**.

The goal of our work in the years ahead is to characterise the genetic, epigenetic and transcriptional changes in prostate cancers. To this end, 250 surgically removed prostate tumours will be prepared and sequenced in comparison with autologous blood samples.

Figure 3: Structure of the ICGC project 'The genomes of early onset prostate cancer'

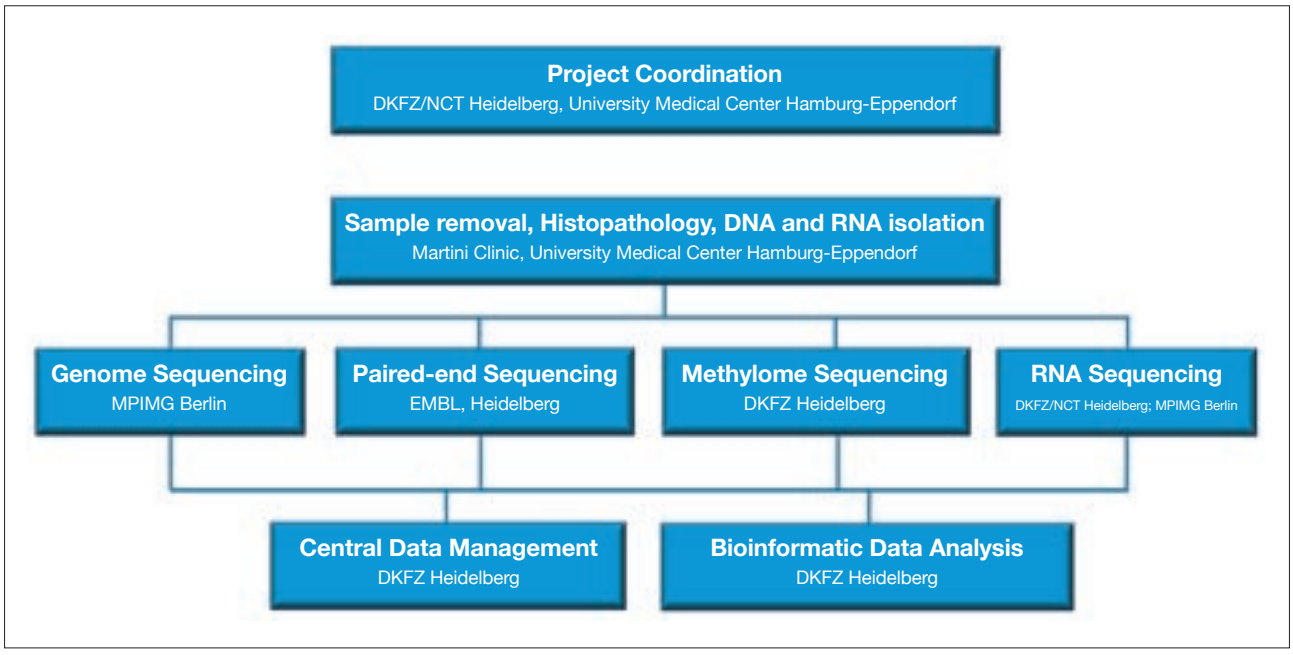


Chart: H. Sültmann

A number of experienced research groups are collaborating closely on this joint, interdisciplinary project (Fig. 3).

In a work process developed specially for the project, the entire prostate is cryopreserved, histologically examined and a highly tumorous area of tissue is selected for the recovery of nucleic acids.

In genome sequencing, specific sample preparation enables the detection of both minor DNA aberrations (e.g. mutations) and bigger structural changes in the DNA. In parallel, methylome, mRNA and small-RNA sequencing is performed on approximately 50% of the same samples.

As a result, in addition to genetic changes, one can detect epigenetic changes and deregulated gene expression along with new transcripts or transcribed fusion genes. Highly specialised integrative data analysis enables the individual levels to be combined so as to obtain information on allele-specific gene expression, for example.

Other international working groups in the ICGC (e.g. in Britain, France, the US and Canada) have set themselves the goal of sequencing the most frequent tumour in men, but they are working on different prostate cancer subgroups. This facilitates a coordinated approach to prostate cancer genome sequencing. In order to benefit from optimum synergy effects from the different projects, we agreed an intensive exchange of experience and data with these groups.

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www.icgc.org/icgc/cgp/70/345/53039

III. ICGC MMML-Seq – Molecular characterisation of germinal centre-derived B-cell lymphomas by sequencing

Lymphomas are malignant tumours derived from immune system cells. The World Health Organisation (WHO) classification distinguishes between more than 40 subgroups on the basis of morphological, clinical and, increasingly, genetic aspects (Swerdlow *et al.*, 2008). More than 13,000 new cases are diagnosed in Germany each year. Among them, Burkitt lymphoma and leukaemia and the diffuse large B-cell lymphomas (DLBCL) are most common in children, and the DLBCL and follicular lymphomas in adults. The ICGC MMML-Seq project focuses on the sequence analysis and cataloguing of genetic and epigenetic changes in these three groups of germinal-centre B-cell lymphomas, which account for more than 80% of all B-cell non-Hodgkin's lymphomas in children and more than 50% in adults (The Non-Hodgkin's Lymphoma Classification Project, 2005; Burkhardt *et al.*, 2005). The aim of the network is to find new starting points for molecular understanding as well as for the classification, the risk assessment and a targeted therapy of these tumours.

In the context of the ICGC, the MMML-Seq project is continuing in the German tradition of investigating the molecular mechanisms of lymphoma development in implementing into lymphoma classification that began in 1974 with Prof. Lennert's 'Kiel classification of lymphomas'. Technological advances revealed new insights into the biology of germinal centre-derived B-cell lymphomas in which German scientists played a part, for example in the context of the joint project 'Molecular Mechanisms in Malignant Lymphomas (MMML)', funded by German Cancer Aid (DKH) between 2003 and 2011. In collaboration with clinical study groups (DSHNHL, NHL-BFM) the MMML consortium succeeded in defining the molecular mechanisms of known (Burkitt lymphoma) and new lymphoma subtypes (intermediate lymphomas between Burkitt lymphoma and DLBCL, IFR4-translocation-positive germinal centre-derived lymphoma in children and young adults), identifying prognostic markers (MYC gene translocations in DLBCL), and in the first genome-wide analysis of the DNA methylation pattern in mature aggressive B-cell lymphomas (Hummel *et al.*, 2006; Klapper *et al.*, 2008a, b; Martin-Subero *et al.*, 2009; Salaverria and Siebert, 2011; Salaverria *et al.*, 2011).

With funding from the German Federal Ministry of Education and Research (BMBF), the ICGC MMML-Seq project is complementing the established structures of the MMML project in clinical trials, reference pathology, gene expression/genetics

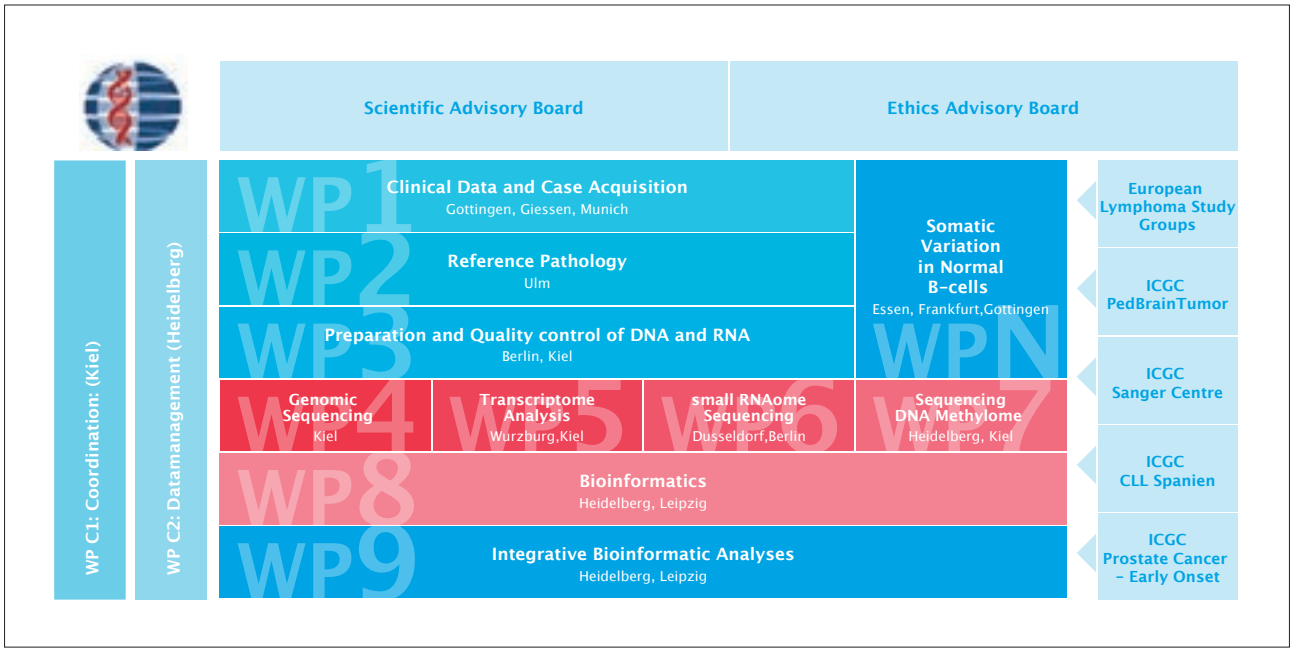

Figure 4: Overview of the ICGC MMML-Seq research consortium

In early 2011, research groups working in the field of lymphoma research and genome analysis, including groups from the universities of Berlin, Düsseldorf, Essen, Frankfurt, Gießen, Göttingen, Kiel, Leipzig, Munich, Ulm and Würzburg, from the German Cancer Research Center (DKFZ) and the European Laboratory for Molecular Biology (EMBL) in Heidelberg came together to form the interdisciplinary research consortium ICGC MMML-Seq. Its aim is to identify somatic genetic changes in tumour cells at the single-base level in order to contribute towards new strategies for the diagnosis, risk stratification and therapy planning of germinal centre-derived B-cell lymphomas and thereby towards the optimal treatment of the patients affected (Chart: R. Siebert).

and bioinformatics with genome-wide sequence analysis at the DNA, RNA and epigenome level (Fig. 4).

In the years ahead, the group will analyse 250 germinal centre-derived B-cell lymphomas and corresponding germline materials by means of standardised sequencing in accordance with ICGC guidelines. In addition, it will analyse B-cell populations in healthy donors in order to characterise the normal changes that take place as B-cells mature in the germinal centre. The analysis includes genome (DNA level), transcriptome (RNA level), small RNAomes (RNA level) and DNA methylation. In addition to data storage, statistical analysis will be undertaken with regard to lymphoma onset. For detailed information on the consortium structure, inclusion criteria and analysis methods, visit www.icgc-lymphoma.de.

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next-generation sequencing

New opportunities in biomedical research

by Stephan Wolf

"DNA sequencing is the determination of the nucleotide sequence in a DNA molecule. DNA sequencing has revolutionised the biological sciences and ushered in the era of genomics …"

These are the opening words in the detailed Wikipedia description (in German) of this rapidly developing technology that can no longer be imagined as anything else but an integral part of molecular biology and medical research.

Since Allan Maxam, Walter Gilbert and Frederick Sanger made the sequencing of DNA molecules technically possible in the mid-1970s, progress in this area has been incredible. On the basis of the revolutionary research findings for which Sanger was subsequently awarded the 1980 Nobel Prize in Chemistry along with Walter Gilbert and Paul Berg, in just a few years a fast-paced technology has developed with which only the most state-of-the-art and innovative laboratories and research facilities can now keep pace.

The history of genome sequencing

The first fully decoded genome in 1977 was that of the bacteriophage φX174. Its circular genome, comprising a mere 5,386 base pairs, was fully sequenced by Sanger using the method that bears his name (Sanger *et al.*, 1977). The full sequencing of the lambda phage followed in 1982, and in 1996 the genome of the baker's yeast *Saccharomyces cerevisiae* was the first eukaryote genome to be sequenced in full.

After the launch of the Human Genome Project (HGP) in 1990 with the aim of fully sequencing the human genome with its more than three billion base pairs, it took over ten years to actually achieve this objective. Not until 1999 was the first human chromosome, chromosome 21, fully sequenced. At almost the same time as Celera, the company founded by Craig Venter, the HGP employees finally announced in 2003 that the human genome had finally been fully "decoded." At the outset, more than 1,000 scientists from around the world took part in the enormous Human Genome Project, and together with Celera spent over USD 6 billion to reach this goal.

Today's state-of-the-art sequencing systems can read a complete human genome in four days using resources to the value of around EUR 6,000 ... and the costs are continuing to fall! Within the next few years we will be able to buy the full sequence information about our own genome for a lot less than USD 1,000, including evaluation and interpretation according to the latest research.

However it is not just human genomes that are analysed using the new systems, but also those of animals, plants, fungi, bacteria and viruses. Since the introduction of the first high-throughput sequencer in 2005, research groups around the world have decoded the genomes of more than 800 species. They range from *Apis mellifera*, the honeybee, to *Yersinia pestis*, the plague virus, and include many different organisms. Everything is sequenced, including primates such as the rhesus monkey and the orang-utan.

"It's neat, let's sequence it! Taste good? Let's sequence it!" was one of the statements made by a Chinese scientist at one of the most important conferences on next-generation sequencing in the US. Many thousands of bacteria genomes and several hundred non-human eukaryote genomes are currently being sequenced.

The 1,000 Genomes Project, the International Cancer Genome Consortium (ICGC) and diagnostic sequencing

Right now, several worldwide projects to sequence human genomes are underway. Since 2008, 2,500 human genomes from 25 different populations have been undergoing sequencing as part of the 1,000 Genomes Project. The aim of this project is to identify genetic variance and the structural and biomedically relevant differences between different individuals and demographic groups.

Stephan Wolf heads the High Throughput Sequencing Unit at the German Cancer Research Center (DKFZ) in Heidelberg (Photo: DKFZ, Heidelberg).

Together with the results of ICGC projects, this data will form the basis for the personalised treatment of a wide range of illnesses. As part of the ICGC, currently one of the world's largest interdisciplinary biomedical projects, the most frequently occurring types of tumour are being sequenced at 39 locations in 13 countries (cf. ICGC article on page 22 of this issue). At present, storing the sequence data of a human genome takes up more than 200 gigabytes of disk space. Storage capacities in the petabyte range are soon required for large-scale projects of this kind, and they are figures with 15 zeros!

On the basis of this data, the development of highly specific, highly effective drugs with few side effects can and is being taken forward much faster and more effectively than previously. Scientists hope to very soon develop tailor-made drugs for many different kinds of cancer.

HiSeq, SOLiD, FLX, Personal Genome Machine…

… are the brand names of the latest sequencing systems. The modern high-throughput sequencing systems that are currently most successful and most frequently used around the world are sold by Roche, Illumina and Life Technologies. Based on different molecular biology and biochemical approaches, all the systems are able to sequence simultaneously and in parallel many – very many – individual fragments. The most powerful system sold by industry leader Illumina, the HiSeq

The High Throughput Sequencing Unit at the DKFZ in Heidelberg

back row, from left to right: Andrea Waxmann, Ute Ernst, Christopher Previti, Sabine Schmidt, Berit Haldemann, Michaela Schanne, Bärbel Lasitschka, Andreas Hunziker, Angelika Ott-Hartmann; front row, from left to right: Nicolle Diessl, Stephan Wolf, Andrè Leischwitz, Andre Götze; not pictured: Nadine Wehran and Ulrike Steck (Photo: Ulrike Conrad).

Part of the next-generation sequencing systems at the DKFZ (Photo: Ulrike Conrad).

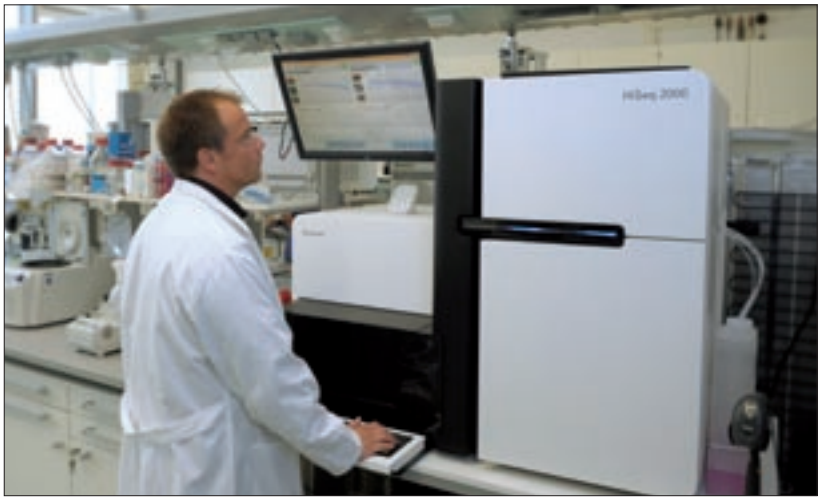
2000, sequences more than 800 million individual DNA fragments of up to 200 base pairs in length in a single session. These individually sequenced, short DNA sections are then reassembled into an overall genome sequence by means of special analytic algorithms for bioinformatic evaluation. This makes it possible to decode up to five human genomes in a single sequencing session. What took years and cost billions before the millennium can now be done in a matter of days and for just a few thousand euros.

And the next generation of sequencing systems, the next-next-generation or third-generation sequencers, are already in their starting blocks. Pacific Biosciences, Oxford Nanopores and IBM/Roche are just three of the contenders for the title of future market leader in this fiercely contested segment of biomedical research. These systems generate much more comprehensive sequencing information even faster and more precisely. Informatics and bioinformatics, as the infrastructure for storage and algorithms for the valid analysis of these immense quantities of data, face the almost impossible task of translating this flood of information into serviceable, diagnostic approaches and effective therapies as fast as possible.

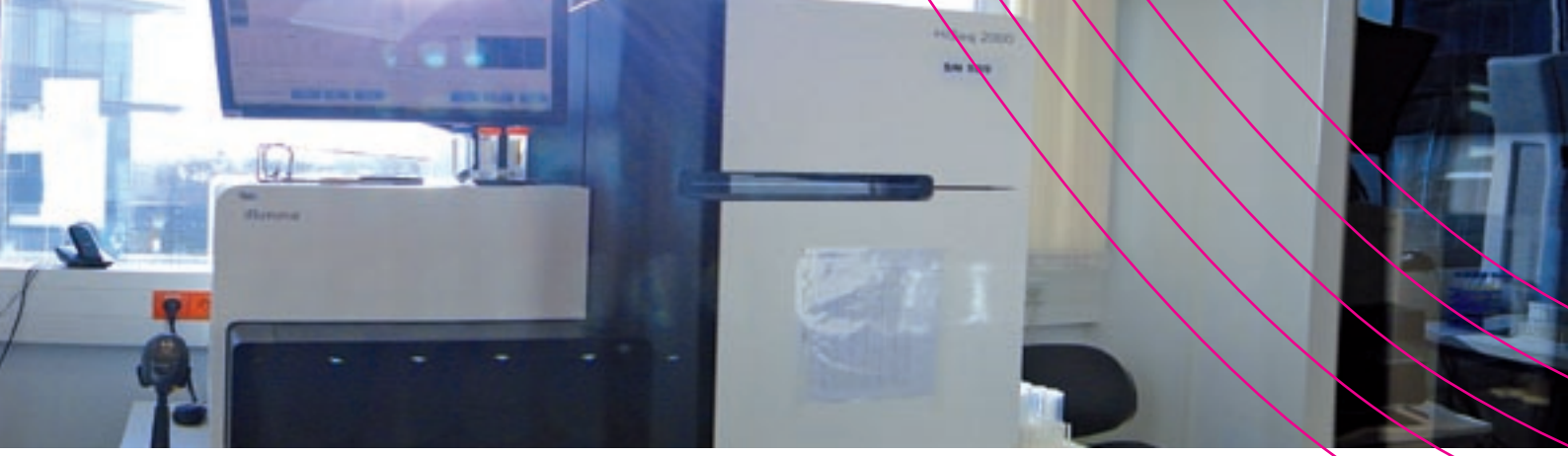
Sequencing facilities

The technical complexity and the substantial, cost-intensive equipment required, coupled with the immense dynamism of this rapidly developing technology, make it economically almost impossible for a single research group to compete and keep up with the pace of development. That is why more and more expertise and equipment at medium-sized and larger research institutions is bundled at so-called core or special-sequencing facilities. These highly specialised central units provide research scientists and groups with access to instruments, technologies and services. It is the only way in which to make efficient and economic use of these high-grade analysis systems. At the National Institutes of Health (NIH), the foremost biomedical research facility in the US, for example, an estimated USD 900 million is spent on operating the core facilities. The world's largest sequencing unit is at the Beijing Genomics Institute (BGI). It has more than 120 of the most up-to-date sequencing systems and is currently involved in several international genome-sequencing projects. Over the next ten years the BGI is due to receive a total of USD 1.5 billion in funding from the China Development Bank, a state enterprise in the People's Republic of China. Since it was founded in 2007, the institute has registered nearly 250 patents in connection with genome sequencing.

Genome sequencing: now in just a matter of days



The Illumina HiSeq 2000 is currently the world's most widely used next-generation sequencing system (Photo: DKFZ Heidelberg).



The largest sequencing unit in Europe is in the UK at the Sanger Institute in Hinxton, Cambridgeshire. Founded in 1992, the largest biomedical research foundation in the world plays a leading role in genome sequencing in Europe.

Core Facilities at the German Cancer Research Center (DKFZ)

The bundling of resources and the setting up of core facilities has for several years been an increasingly important consideration at the German Cancer Research Center in Heidelberg. Around 800 researchers at the DKFZ have a total of six different, highly specialised core facilities at their disposal. From imaging and cytometry, from information technology to genome and proteome research, the different units serve nearly all areas of modern cancer research. The expertise concentrated at the DKFZ and reliably available here enables scientists to concentrate on their core concerns: Cancer research, cancer diagnostics, and cancer therapy.

At the sequencing unit, which was set up in 2010, more than 70 scientific working groups have access to the most technically advanced systems as well as the expertise of a total of 15 scientists, bioinformatics experts, engineers and technicians. Seven HiSeq 2000 systems, several SOLiDs, a Roche-454 system and other, smaller platforms are available here, making the unit one of the largest in Europe. The sequencing unit sees itself not just as a service provider and enabler for established research approaches and protocols, but also endeavours, together with the different research groups, to develop and implement innovative ideas and new technologies. Experiment design and implementation, and the evaluation and interpretation of the findings are supported at the sequencing unit. Along with the other “core family” units, DKFZ research scientists have a sound, consolidated infrastructure at their disposal to enable them to remain internationally competitive and to focus on measurable improvements in the diagnosis and treatment of cancer. The DKFZ sequencing unit makes substantial contributions to the international ICGC PedBrainTumor and prostate cancer projects (see page 22 of this issue) and is the sequencing basis for the new Heidelberg Initiative for Personalised Oncology.

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using systems (biology) to tackle brain tumours

The Slovenian-German SYSTHER Project

by Kathrin Jürchott, Michal Or-Guil, Christian Schichor, Johannes Schuchhardt and Joachim Selbig

The binational SYSTHER Project, or "Systems Biology Tools Development for Cell Therapy and Drug Development", is funded by the German Federal Ministry of Education and Research and is run under the auspices of the Slovenian-German research initiative INREMOS. The aim of INREMOS, or "Industry-Relevant Molecular Life Sciences", is to advance the transfer of technology and encourage the foundation of start-up companies. In the case of SYSTHER, the participating groups in Germany have concentrated since 2007 on advancing the understanding of brain tumours. Clinical, experimental and theoretical study groups are jointly developing new approaches to therapy and to minimally invasive procedures for diagnosing this type of tumour.

Brain tumours as a model

Despite intensive research and a range of treatment methods (operation, radio- and chemotherapy), malignant brain tumours (glioblastomas) are generally incurable. This is not, as with other types of tumour, due to the formation of metastases. Rather, despite radical operation the glioblastoma tends towards recurrent growth, beginning with tumour cells that have dispersed into the surrounding healthy brain and which, even with modern diagnostic methods, cannot be identified or properly treated. The goal of the SYSTHER Project, which has been underway since 2007, is to contribute towards a better understanding of molecular processes during the onset and growth of a tumour, thereby laying the foundations for new therapeutic approaches and improved diagnostics. The project takes a systems biology approach. In other words, it is not interested in individual proteins, as was customary in the past, but primarily in their interactions. That is because it is now clear that the causes of disease-based changes in cells that lead to the onset of tumours do not lie in a single gene but in the interplay between various molecules in cells.

Metabolism and tumours – learning from plant research?

As early as the 1920s, Otto Warburg examined the metabolism of tumour cells and discovered that, in contrast to normal cells, they obtain most of the energy carrier ATP via glycolysis, even in aerobic conditions, that is, when sufficient oxygen is available.

Research into metabolic processes in tumours has experienced a steep upturn in recent years and developed into a new focus in cancer research (Cairns *et al.*, 2011). With the help of modern methods such as GC/MS – mass spectrometry coupled with gas chromatography – it is possible to study the metabolism of cells and tissues. With this method, several hundred small molecules known as metabolites, which are intermediate and end products in metabolic processes in cells, can be identified in parallel in a single measurement, thereby producing a snapshot of metabolism. Medical research can use methods that have long been applied in plant research and are undergoing constant further development. In SYSTHER, too, we benefit from this experience and work closely with experts at the Max Planck Institute for Molecular Plant Physiology in Potsdam.

Within the framework of SYSTHER we study metabolism in tumour cells and in normal, non-transformed cells from the tumour's surroundings. By analysing this data we aim to identify significant differences between the two groups, thereby finding an Achilles heel in the metabolism of cancer cells, on the basis of which new therapeutic approaches can be developed. Our investigations to date have already identified a number of clear differences in fundamental, essential metabolic pathways such as the citric acid cycle and glycolysis. Evidently, inside and also outside these metabolic pathways individual metabolites have a specific correlation with others. These correlations can be used to construct networks that visualise the relationships between the metabolites and provide the opportunity to apply mathematical methods, in particular graph theory methods, to data analysis (Jürchott *et al.*, 2011). Figure 1 shows correlation networks of this kind for stem cells and brain tumour cells.

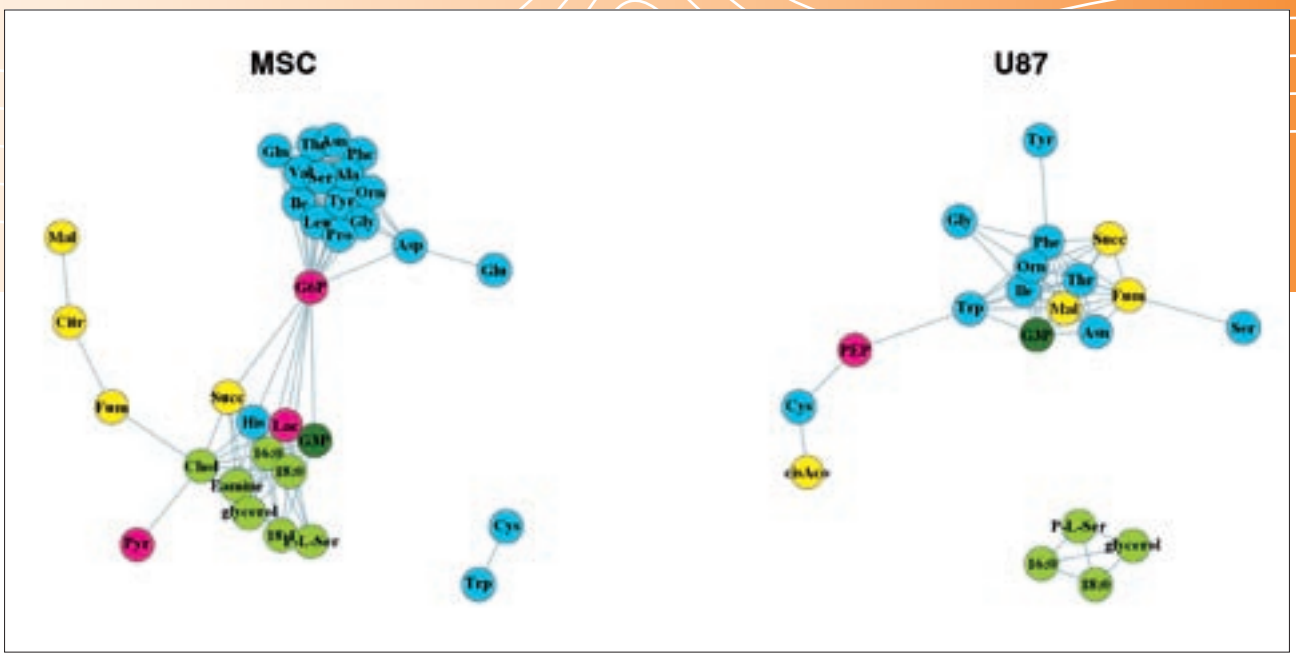


Figure 1:

The correlation networks of human mesenchymal stem cells (left) and U87 glioma cells (right). Clear differences can be seen in the relationships between groups of metabolites, which are colour-coded (yellow: citric acid cycle, red: glycolysis, blue: amino acids, green: lipids) (Source: Jürchott *et al.*, 2011).

Tumours and the micro-environment

A further focus of the project is on the interaction between tumour cells and host cells. Tumour tissue is heterogeneous and, along with tumour cells, also consists of normal, non-transformed cells. The function of these normal cells, which may even support the tumour, is not known. Both types of cell, tumour cells and normal, non-transformed host cells, are dependent on the surrounding micro-environment, but probably modulate it, too, in order to achieve optimal conditions for the tumour to grow. Consequently, understanding the complex interrelationships in this system is an important basis for developing new medicines and therapeutic approaches, and is the subject of intensive research.

Research in the SYSTHER project is focused especially on mesenchymal stem cells (Motaln *et al.*, 2010). These cells are precursor cells in the supporting and connective tissue and they are important for the regeneration of bone, cartilage, muscle, ligaments, tendons and fatty tissue. Experiments have shown that mesenchymal stem cells are attracted by the tumour, migrate into the tumour tissue and interact with tumour cells. As a result of the interactions, these cells change. The processes involved are described as "instructive reprogramming". Within the framework of the project we use cell cultures to investigate which signals control the migration of stem cells to the tumour. We were able to show that glioblastomas secrete a number of angiogenic cytokines designed to encourage blood circulation and vascularisation in the tumour, and thereby also actively direct mesenchymal stem cells to the tumour. Figure 2 shows a cell culture experiment of this kind. In the mixed cell conglomerate (spheroid), tumour cells and stem cells influence each other's migratory behaviour.

In order to better understand the processes of reprogramming stem cells through interaction with tumour cells, we are currently engaged in the intensive analysis of gene expression profiles. To model such processes we use flow coupling analysis (FCA) and structural kinetic modelling (SKM).

Phenotypic analysis of immune response and serum profiling

Cancer cells are genomically unstable and accumulate more and more mutations as cells divide. Changes in the genetic material may give rise to new, extraneous gene products and proteins known as tumour antigens, which trigger an immune reaction when they are present on the cell surface.

The systems immunology study group at Berlin's Humboldt University is investigating the immune response of B-cells. Researchers use flow cytometry, immune histology and molecular biology methods to characterise antigen-specific B-cells at the cellular and tissue level. They focus on the kinetic investigation of the

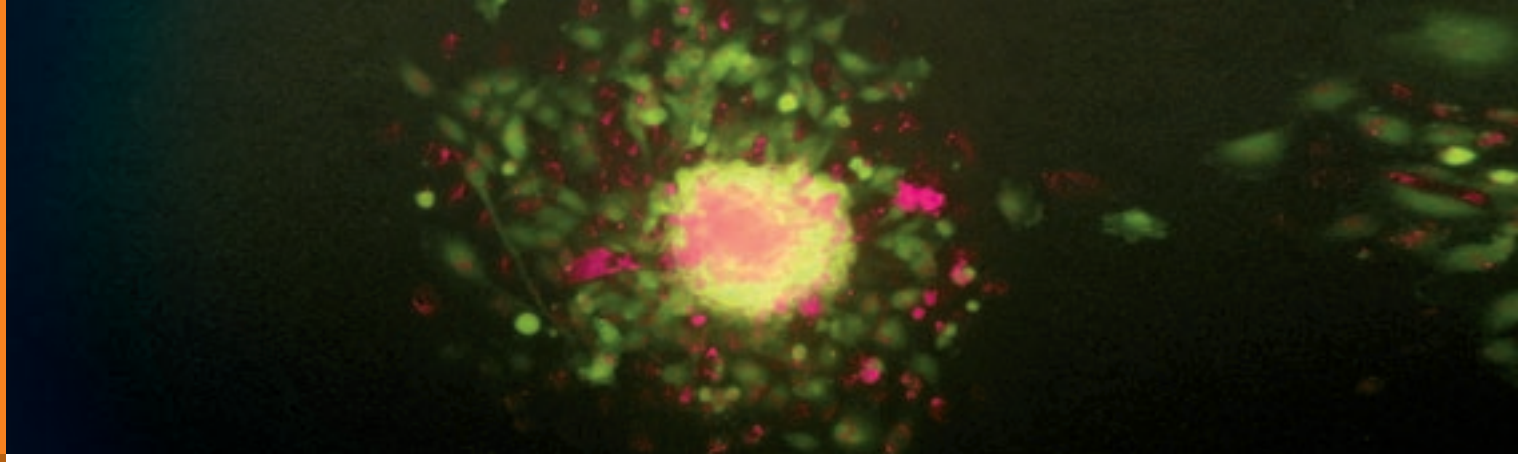


Figure 2: Tumour cells (green) and stem cells (red) reinforce each other's migratory behaviour in a cell culture. The image shows a mixed cell conglomerate (spheroid) (Source: C. Schiror, Neurosurgical Clinic, University Hospital Großhadern, Ludwig-Maximilians-Universität Munich).

localisation and migration of B-cells during the course of an immune response. One key function of B-cells is to mediate the humoral immune response (from Latin [h]umor = liquid). After activation, some of them differentiate into plasma cells that produce large quantities of antibodies and pass them to the body fluids. These antibodies then circulate freely and unattached to cells in the blood, lymph and elsewhere. Thus the total sum of all antibodies in blood serum indirectly reflects all antigens recognised by the immune system at a specific time. Accordingly, it should be possible to detect the appearance of new antigens in the serum, for example through the multiplication of cancer cells. Serum profiling is therefore a suitable candidate for minimally invasive methods of tumour diagnosis.

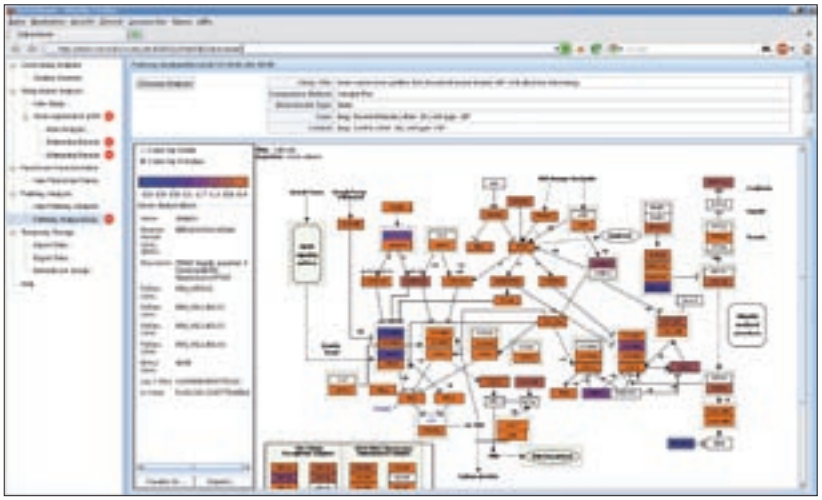
The systems immunology study group is currently concentrating on the issue of minimally invasive methods of tumour diagnosis for gliomas, with the aid of high-throughput analysis of serums from healthy donors and glioma patients. To this end, the serum antibody bindings are measured against random libraries for peptide arrays.

Since kinetic investigations of the B-cell response and the high-throughput profiling of serums generate large volumes of raw data, the group is concentrating in particular on bioinformatic data analysis and management. Thus, in collaboration with MicroDiscovery GmbH we have established a freely accessible database system for immune-histological images and their analysis: www.sysimmtools.eu (Wittenbrink *et al.*, 2011), as well as a tool for determining the clonal relationship of B-cells.

SystherDB – a systems biology-oriented tumour-specific database

In SYSTHER, extensive experiments and studies generate large volumes of diverse data: Gene expression profiles, metabolite data, peptide array data and phenotypic data from clinical studies. To manage this heterogeneous data, we worked with MicroDiscovery GmbH, the Berlin-based specialist in bioinformatics solutions, to set up a modern database which enables the storage of data generated in the project. A Web interface was developed in order to enable user-friendly access to the data, especially for our partners in the project. Our database holds information that has already been processed, which is connected to references and links to important public databases, for example the Ensembl genome browser and the pathway-oriented KEGG database. It supports integrative data analysis whereby, for example, gene expression and metabolite data can be analysed and presented in parallel.

Figure 3: The SystherDB



In this example, the SystherDB shows the expression of genes that are incorporated in the cell cycle. The underlying data comes from an experiment that analyses the effects of dexamethasone, an anti-inflammatory glucocorticoid, on U87-cells. Genes coloured in dark blue, for example the SMAD3 transcription factor, show clear differences in gene expression (Source: Microdiscovery GmbH).

The database is currently used by the partners in the SYSTHER Project. Figure 3 shows one of its many possible applications. Tumour-relevant metabolic pathways and other cellular reaction pathways, in this case the cell cycle, are shown in an interactive graph and overlaid with the results of gene expression analyses which show whether and to what extent genes are expressed differentially.

The research project in brief:

SYSTHER – Systems Biology Tools Development for Cell Therapy and Drug Development – is a binational project on which Slovenian and German groups are working closely together. SYSTHER is funded by the German Federal Ministry of Education and Research.

Participating partners:

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MicroDiscovery GmbH, Berlin, Dr. Johannes Schuchhardt;
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www.systher.eu

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brain research needs a network

Computational and Systems Neuroscience at the Research Center Jülich

by Sonja Grün and Markus Diesmann

Nobody has ever devised a structure like the human brain, yet in the years ahead, supercomputers will become sufficiently powerful to simulate large parts of the brain at the level of nerve cells. It is now up to scientists to collect the necessary data and find a theoretical framework. Global competition on technical applications has already begun, and the Research Centre Jülich has set its sights firmly on the tasks ahead.

Computational neuroscience – a young discipline seeks to understand the brain

Computational neuroscience took shape at the end of the 1980s when scientists, many from the field of theoretical physics, undertook initial research to gain an understanding of the dynamics and function of the brain by means of mathematical models. The brain's heterogeneous and hierarchical structure had made it clear to early anatomists like Ramon Cajal (1852–1934) that information processing is borne by interaction between nerve cells. That is why, if initial statements were to be made about the behaviour of these networks, it was necessary from the start to describe the dynamics of the individual nerve cells in a few equations.

A nerve cell (neuron) maintains an electric potential between its interior and its surroundings. When this potential exceeds a certain threshold level, a sharp voltage pulse, or action potential, is triggered. In the subsequent neurons the voltage changes ever so slightly as a result (Fig. 1A). But one neuron receives input from ten thousand others. If these inputs interfere with one another, the threshold may be crossed again (Fig. 1B). This causes permanent neuronal activity along the anatomical feedback structures. Animals learn new behaviour from the change in coupling strengths at the points of contact (synapses). How the brain sets the right synapses at the right strengths is still largely unknown.

Reduced models as a key to understanding

The threshold value process is hard to handle mathematically, which is why neuroscientists often have to rely on simulations. Comparison with experimental data enables them to check hypotheses on the relationship between network structure and observed activity.

As in other areas of science, describing the system in as much detail as possible does not make sense. Insights are often only achieved by means of models that are mathematically reduced to such an extent that they just account for an observation. Simulation can be used to carry out model reductions in a controlled fashion because results before and after simplification can be compared. With simplified models scientists may succeed in solving equations in such a way as to expose the dependence of an effect on the parameters.

Internationally, computational neuroscience is seen not just as computer-assisted neuroscience but at the same time as a science that investigates how the brain computes. Even though Germany did not get going until about ten years after centres in Israel, Japan and the USA, the national Bernstein Network Computational Neuroscience now does top-level research in this field. A German translation of the term "computational neuroscience" has never caught on. The German term "Neuroinformatik" has been reinterpreted several times and does not refer to a natural science focus.

Systems biology took shape at the end of the 1990s, concentrating at first on processes inside the cell, whereas computational neuroscience focused on interactions between nerve cells.

Although both areas have their origins in 1950s systems theory, they were quick to develop in different directions (De Schutter, 2008). Today they are converging in content and method again because a systemic understanding of the brain requires all scales.

Prof. Dr. Sonja Grün and Prof. Dr. Markus Diesmann head the INM-6 at the Research Center Jülich (Photo: Research Centre Jülich).

A new institute as a link

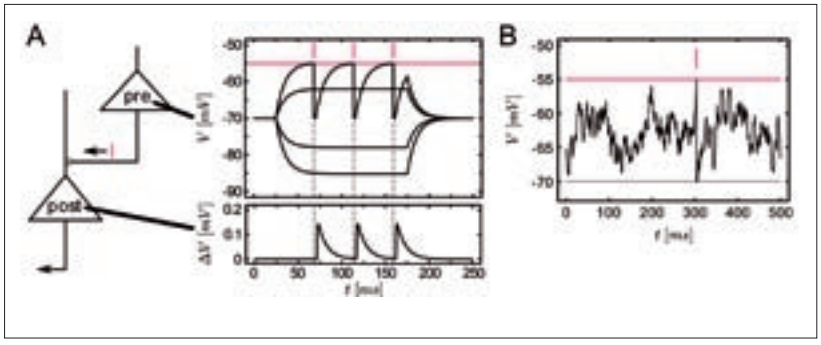
Since 2008, the Jülich Research Centre has been in charge of the Helmholtz Alliance on Systems Biology's Human Brain Model network. Its long-term aim is to draw up a model of the human brain (Diesmann, 2010). Jülich already has expertise in the areas of neuroanatomy, neurophysiology and functional imaging at several institutes. Its Institute for Advanced Simulation (IAS) is one of the leading centres for supercomputing. The establishment of the Institute of Neuroscience and Medicine (INM-6), Computational and Systems Neuroscience, added an institute for theory to the range of methods available and built a bridge.

From March 1st 2011, the INM-6 was filled with life when the Statistical Neuroscience (Grün) and Computational Neurophysics (Diesmann) working groups relocated from the RIKEN Brain Science Institute near Tokyo. A core group in Jülich consisting of Dr. Wiebke Potjans, Dr. Tobias C. Potjans and Dr. Jochen M. Eppler had prepared the transfer, with Dr. Eppler in particular pressing ahead with the construction of the new building. The Tohoku earthquake on March 11th, followed by the tsunami and nuclear catastrophe in Fukushima, had no direct effect on our laboratory. The relocation of personnel, however, did not take place gradually until the end of August as planned, but went ahead within a few days. We helplessly followed events in the country we had just been living in, thought a lot about our colleagues and friends in Japan and admired the discipline with which life and research were restored to normal.

INM-6 – structure and expertise

Computational neuroscience can be extended along two axes. The content axis must cover structure (anatomy) and dynamics (activity) with the second axis covering the level of description. Bottom-up is the term used to describe the approach to understanding the system by interconnecting the smallest components. If, to begin with, a theory of system behaviour is proposed to deduce which combination of structure and dynamics produces the function, this is called a top-down approach. In their extreme forms both approaches must be criticised. Without knowledge of superordinate rules, there can surely never be enough data to adequately limit a model of the self-organised

Figure 1: Fundamental interaction between nerve cells



(A) Two coupled nerve cells (triangles, circle: synapse; red: action potential) and the time course of the membrane potential.

(B) In living animals the membrane potential exhibits large fluctuations. Revised by Susanne Kunkel after Diesmann, 2002, PhD lecture, Ruhr-University Bochum.

Computational neuroscience thrives on communication between scientists undertaken by large, international groups using state-of-the-art technology to coordinate it (Photo: Research Center Jülich).

brain. On the other hand, making deductions as to structure from system behaviour is not necessarily unambiguous.

The **Statistical Neuroscience group** deals with the development of statistical methods to analyse multichannel data from neuronal activity (local field potentials or LFPs, sequences of action potentials). To this end, statistical analysis tools are developed that enable scientists to record time-dependent, behaviour-coupled interactions between large numbers of nerve cells (for an introduction see Grün and Rotter, 2010). The working group aims to make tools and procedures generally available and organises educational programmes and advanced-level courses.

The **Computational Neurophysics group** deals with the construction of mathematical models of brain circuitry. The chosen level of description is that of the nerve cells, which interact with one another via 10,000 points of contact. Simulations provide an insight into the detailed processes that go on in the regenerative and hierarchically structured networks. The group is also engaged in work on the foundations of network theory. The resulting equations create a deeper understanding of the relationship between structure and dynamics. To simulate larger circuits, the group is conducting intensive research into software technology for supercomputers (www.nest-initiative.org). The principal focus of the research group is the bottom-up approach. An example of combining the bottom-up and top-down approaches is the recently published study on the role of dopamine in temporal-difference learning (Fig. 2).

Onwards

In the months ahead our task will be to further integrate the INM-6 (www.csn.fz-juelich.de) into the European research networks. To achieve a balanced coverage of the different areas of theory we aim to extend our expertise in the areas of theoretical neuroanatomy and functional neural circuit theory by means of a top-down approach. Close contacts with the International Neuroinformatics Coordinating Facility (INCF) already exist via our participation in the Multiscale Modeling Programme, the organisation of courses and joint funding of the CoCoMac database (cocomac.g-node.org). Preparations are underway to link Jülich to the Bernstein Network Computational Neuroscience, and in March 2012, INM-6 is hosting the annual conference of the European BrainScaleS project (www.brainscales.org).

The Statistical Neuroscience group is currently working on setting up an electrophysiology laboratory of its own at the CNRS in Marseille. There, our partner Dr. Alexa Riehle (Riehle and Vaadia, 2005) will use the Jülich device to record simultaneously the activity of nerve cells in two areas of the brain via 100 channels in each. Finding the best possible location for a scientific apparatus, at Jülich or elsewhere, is one of the research centre's tried-and-tested strategies.

Beyond Europe we plan, in addition to our links with Israel, to above all maintain our links with Japan and to continue our research at the K supercomputer. Supercomputers are becoming data integration machines. Software development, however, requires a stable technical and personnel infrastructure over a longer period. Based on the mission of the Helmholtz Association, Jülich is in a position to provide this.

Developing simulation technology is a major challenge. It is no less important, however, to find out how a simulation tool can be tested for accuracy in practice and how results can be published in a reproducible form (for further details see Diesmann and Lanser, 2012). In connection with this, a cultural problem arises. Models are frequently developed by individual working groups and not combined into larger units using modules from other groups, but the heterogeneous structure of the brain with its many subsystems will force us to learn to build on the work of others.

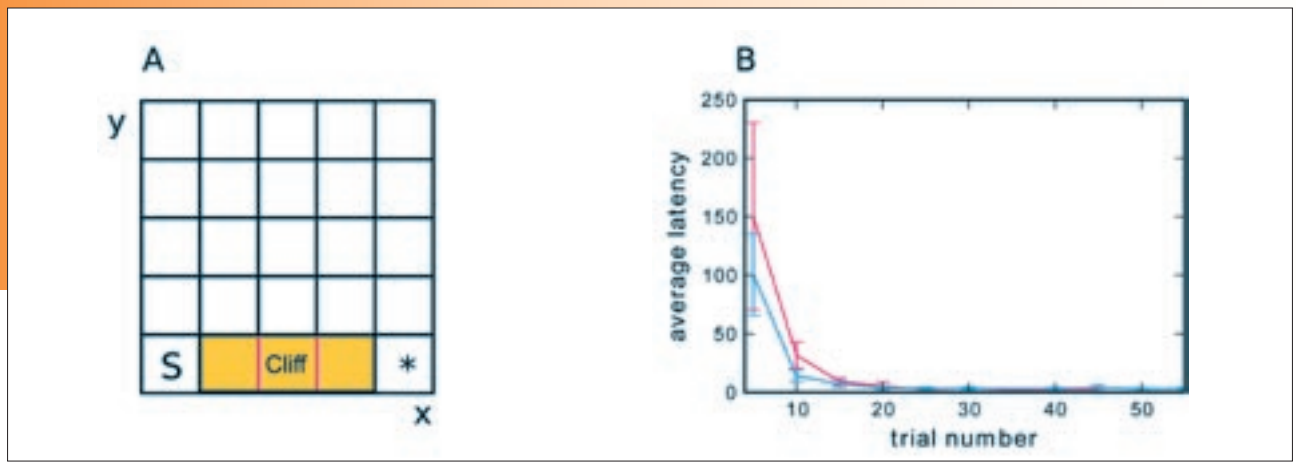


Figure 2: Top-down and bottom-up approach combined

In a grid-world (A) a system learns how to bypass a cliff (yellow) and reach its destination (asterisk) from the start (S). (B) A neuronal network (red) controlled by dopamine solves the task almost as quickly as is theoretically possible (blue). Compiled from Potjans *et al.* (2011).

The research described is heading towards networks on the scale of a brain and thereby appears to be parting company with systems biology. In fact, however, many scales need to be taken into consideration simultaneously (Noble, 2008; Tretter *et al.*, 2010). The synaptic plasticity on which system-level learning is based and the new field of optogenetics are cases in point.

Computer technology is helping us to constantly improve our models. Knowledge about how the brain functions will lead to new medical applications. The architecture of the brain is, however, totally different from that of today's computers and its energy efficiency in certain tasks is unrivalled. A further benefit of computational neuroscience – the development of novel computer systems based on the principles of the brain – is on the horizon.

The research project in brief:

INM-6 Computational and Systems Neuroscience is part of the Jülich Research Centre's Institute of Neuroscience and Medicine. It was established in 2009 with assistance from the Helmholtz Alliance on Systems Biology. Prof. Karl Zilles first headed it provisionally. Since 2011, Prof. Markus Diesmann has been INM-6's director and Prof. Sonja Grün its permanent deputy director.

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LungSys – the systems biology of lung cancer

Risks of erythropoietin treatment in lung cancer and predicting prevention strategies

by Ursula Klingmüller, Julie Bachmann, Sofia Depner, Agustin Rodriguez, Marcel Schilling and Michael Thomas

Throughout the world, lung cancer has one of the highest mortality rates of any cancer. Patients are often diagnosed at a very late stage when the disease is already far advanced and chemotherapy is needed. In the course of this therapy, patients may develop anaemia, which can be treated with the hormone erythropoietin (EPO). However, since quite some time the safety of this treatment has been discussed controversially because clinical studies had to be terminated due to undesired side effects. To unravel the multi-level effects of EPO in cancer, we apply a systems biology approach that facilitates an analysis of the dynamic interplay between different system components and the prediction of strategies for targeted intervention.

Every year approximately 7.4 million people die of lung cancer and that number is predicted to rise to nearly 12 million by 2013 (www.who.int). This makes lung cancer one of the deadliest forms of cancer that accounts for 13% of all cancer-related fatalities. The most common type of lung cancer is non-small-cell lung carcinoma (NSCLC). The absence of symptoms during the early stages of the disease and early metastasis are the most common reasons why this type of cancer is usually identified very late when it is already far advanced. Only in rare cases patients with metastatic lung cancer are still alive

Figure 1: Schematic depiction of the LungSys consortium

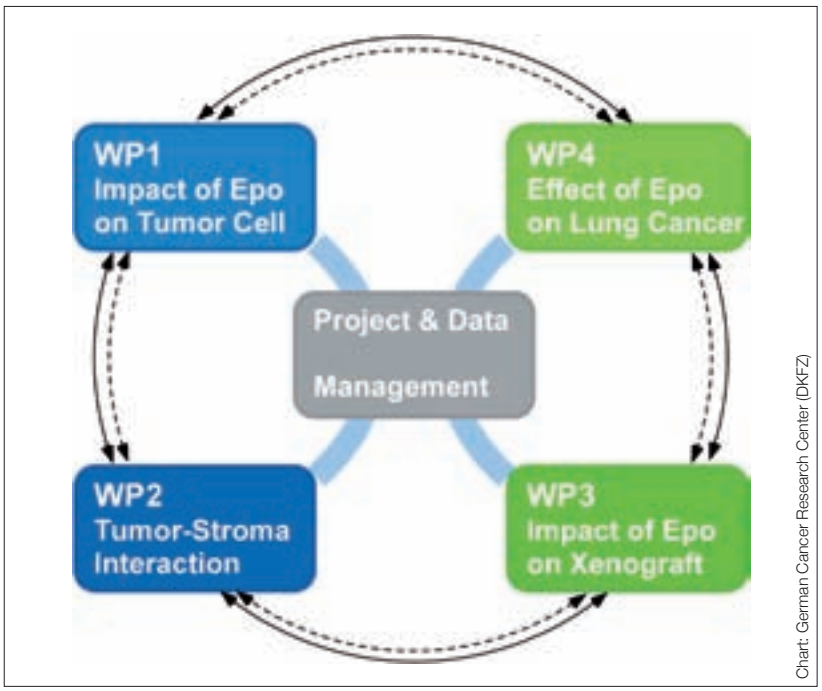

Chart: German Cancer Research Center (DKFZ)

The LungSys consortium is structured in four work packages (WPs). The first, WP1, addresses the characteristics and function of EpoR in lung cancer cells. In WP2 interactions between tumour cells and cells in the tumour micro-environment are analysed. Researchers in WP3 use small-animal tumour models to enable a better understanding of the effects of EPO on tumour tissue. WP4 is concerned with a risk-benefit prediction for EPO therapies in lung cancer patients.

five years after diagnosis. One therapeutic and palliative treatment for advanced lung cancer is chemotherapy. As a side effect of this treatment patients often develop anaemia, which decreases their quality of life significantly and makes blood transfusions necessary. In order to counteract anaemia, erythropoietin (EPO) is administered to stimulate the formation of red blood cells (erythrocytes). EPO induces the production and differentiation of erythrocytes in bone marrow and restores a normal level of oxygen supply to the tissue. However, the safety of this treatment is the subject of controversy since clinical studies have showed a decline in the progression-free survival of patients treated with EPO.

Although the detailed molecular mechanisms are yet unknown, EPO is thought to influence the behaviour of cancer at several levels. The Systems Biology of Lung Cancer consortium (LungSys, www.lungsys.de), funded by the German Federal Ministry of Education and Research (BMBF), is therefore applying an integrative mathematical modelling approach in order to better assess the risks of EPO treatment in lung cancer patients and to enable the optimisation of personalised therapy.

The project partners

Partners from a variety of disciplines are collaborating closely in the LungSys consortium (Fig. 1). Biologists (U. Klingmüller, M. Müller), physical chemists (D.-P. Herten), medical physicists (F. Kiessling), clinicians (M. Thomas, N. Reinmuth, H. Hoffmann, C. Heußel, H.-U. Kauczor), bioinformatics experts (R. Eils, H. Busch), biostatisticians (F. Theis), a biophysicist (T. Höfer), theoretical physicists (J. Timmer, H. Busch, D. Drasdo), a biomechanical engineer (I. Vignon-Clementel), the pharmaceutical industry (Roche Diagnostic) and medical diagnostics specialists (MeVis research/ MeVis Medical Solutions) are enabling the quantitative analysis of the effects of EPO on tumour growth and angiogenesis from the single-cell level to the patient. In an iterative process combining quantitative data generation and mathematical modelling the characteristics of the system are identified and used to establish biomarkers that make it possible to assess the risks to patients undergoing EPO therapy.

The effects of EPO in lung cancer cells

EPO is the main regulator of the formation of red blood cells (erythropoiesis) and binds to a cell-surface receptor that is present on erythroid precursor cells and was recently discovered to be present on cancer cells and endothelial cells, too. Therefore, a key question is whether the EPO receptor (EpoR) on cancer and endothelial cells has similar dynamic properties as in the erythroid system and whether the transmission of signals is different. In erythroid cells we were able to show that ligand-independent turnover of the EpoR and rapid breakdown of the ligand plays a key role in the EpoR signalling system and enables linear signal conversion for a broad range of EPO concentrations (Becker *et al.*, 2010) (Fig. 2). These findings and the developed mathematical model provide a basis for the analysis of the characteristics of the EPO-EpoR system in lung cancer cells. Thereby we could already successfully establish the dynamic parameters for the interaction of EPO with EpoR in the context of lung cancer. Furthermore, in primary erythroid precursor cells, with the help of mathematical modelling, we were able to show a linear correlation between the integral response of STAT5, an important intracellular signalling protein of the EpoR, and the survival of erythroid cells (Bachmann *et al.*, 2011). By linking the two mathematical models and adjusting to the dynamics of EPO-induced signal transmission in lung cancer, we were able to make some initial predictions about differential effects of EPO. We are also in the process of integrating the influence of microRNA regulatory networks and, with the help of single-molecule spectroscopy and live cell microscopy, examine the dynamics of fluorescently labelled signalling components at the level of individual cells to gain a deeper understanding of differences in the haematopoietic system and in lung cancer cells.

Effects of EPO on the tumour stroma and on angiogenesis

The specific effects of EPO on the tumour micro-environment are analysed by means of comparative modelling in endothelial cells and fibroblasts. The main focus of these studies is on changes in the tumour stroma. Angiogenesis in the tumour is

Image: © Sebastian Kaulitzki – Fotolia.com

tracked over time in three-dimensional co-culture experiments by means of histology and microscopy, and quantified in a xenograft lung cancer model by employing functional imaging. We succeeded in developing a highly sensitive near-infrared (NIR) EpoR probe for fluorescence-mediated tomography (FMT) (Doleschel *et al.*, 2011) that enables us to examine the behaviour of EpoR-expressing tumours in cell culture and in animal models (Fig. 3). On the basis of this data we are now developing a cell-based multi-scale model for the spatial and temporal organisation of tumour angiogenesis and EPO-mediated changes.

Clinical application

Since the EPO receptor was also discovered on endothelial cells, and angiogenesis inhibitors are increasingly used in lung cancer therapy, we are examining the hypothesis that EPO could influence angiogenesis in tumours. In the clinic we are therefore investigating surrogate angiogenesis markers in lung cancer patients who are undergoing chemotherapy either in combination with EPO, or without. For these tests, it is essential to develop suitable, non-invasive tools. An initial success was our development of an NIR-EpoR probe (Doleschel *et al.*, 2011) on the basis of which we are well advanced in developing a positron emission tomography (PET) probe. This probe will facilitate to trace EpoR-positive lung cancer tumours in patients and to observe them non-invasively. In order to generate quantitative data for mathematical modelling from these tests, a special computer program was developed. The resulting information will be used to develop a detailed multi-scale model. To strengthen the predictive power of this multi-scale model, we will integrate the dynamic models of the signalling pathways

Figure 2: Dynamic model of the EpoR system in erythroid cells and analysis of EpoR-positive tumours in an animal model

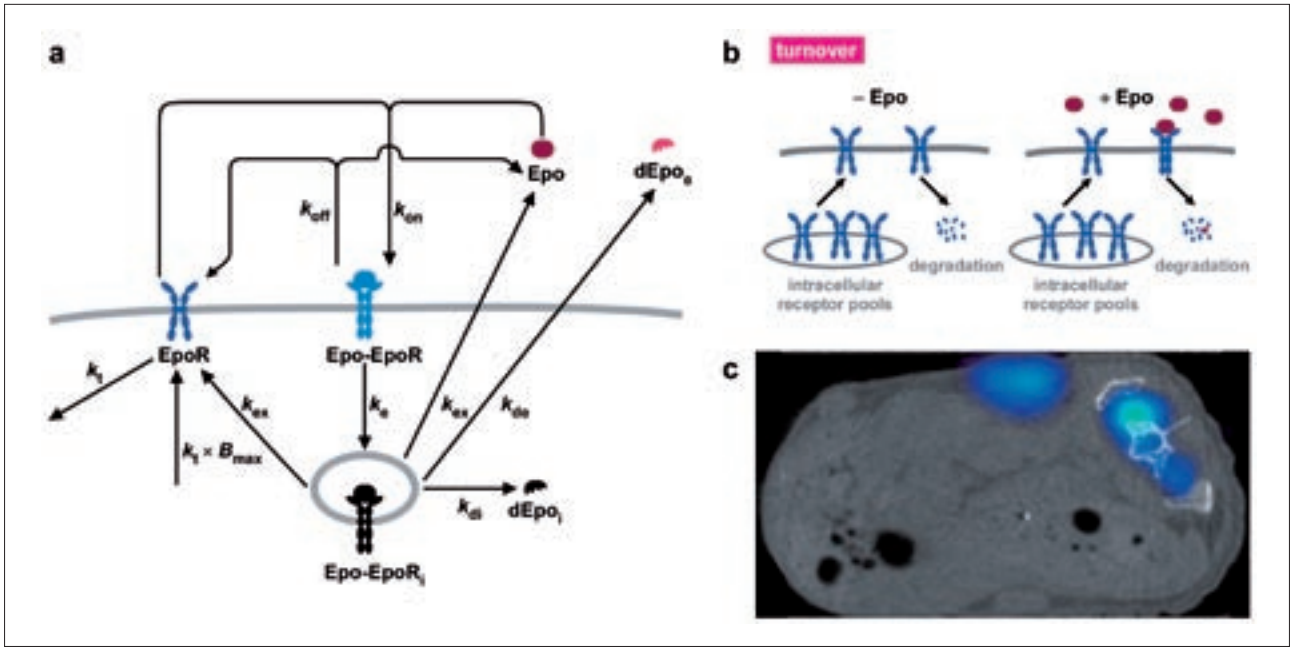

Chart: German Cancer Research Center (DKFZ)

a) Scheme of the mathematical model for EpoR turnover and recovery.

b) Rapid EpoR turnover on the cell surface enables recognition of the EPO signal for a broad range of concentrations.

c) The marked EpoR probe (blue signal) is used for the *in vivo* analysis of EpoR-positive tumours.

that map the effects of EPO at the cellular level. The goal is to use integrative mathematical modelling to stratify patients concerning risks as a reaction to EPO treatment and use model-based fingerprinting to identify prognostic biomarkers.

LungSys II

By focusing on the effects of EPO in lung cancer, a very efficient and successful collaboration between theorists, chemists, biologists and clinicians was established within the LungSys consortium. For the recently started BMBF-funded consortium "LungSys II – Systems Biology of Lung Cancer – Dynamic Properties of Early Spread and Therapeutic Options", this provides an important basis to address one of the key problems of lung cancer, namely the early and systemic spread of tumour cells regardless of the size of tumour. Until now, therapies involving low-molecular inhibitors or therapeutic antibodies frequently have only transient effects due to secondary mutations, since extensive interactions between the signal transduction of growth factors and other signalling pathways exist. Integrative mathematical modelling makes it possible to quantitatively identify the complex dynamic interplay between different system components and to predict the effect of disruptions by, for instance, combination therapies. This approach holds the potential for both optimising therapy options and for adapting them to the requirements in individual patient and thus promises to make significant contributions towards personalised medicine (Fig. 3).

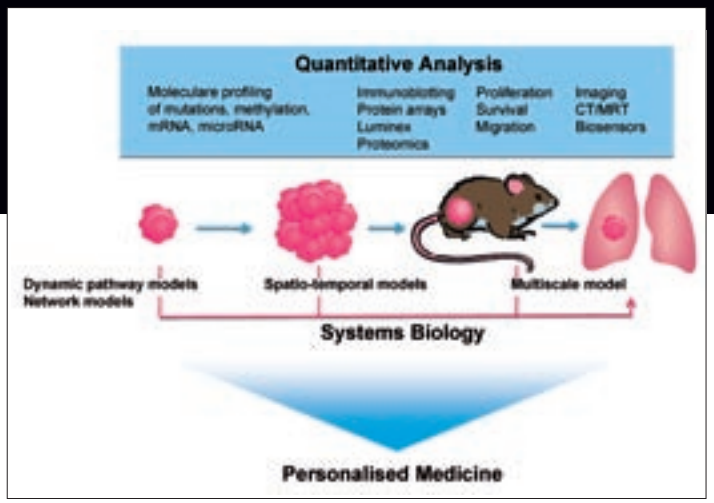

Chart: German Cancer Research Center (DKFZ)

Figure 3: The systems biology of lung cancer and the outlook for personalised medicine

Quantitative analysis of changes in a tumour at the level of individual cells up to the organ, combined with integrative mathematical modelling, will make it possible to identify predictive biomarkers and to optimise therapy options for patient subgroups.

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how skin cells become liver cells

New ways to reprogramme somatic cells

by Max Flöttmann, Till Scharp, Ying Wang, Katharina Drews, Xinlai Cheng, Stefan Wölfl, Alexander Hahn, Sheraz Gul, Nancy Mah, Miguel A. Andrade-Navarro, Edda Klipp, Gunter Wolf, James Adjaye and Ralf Mrowka

Pluripotent stem cells are the true all-rounders among cells. They can change into any other kind of cell and are therefore highly valuable for research and for future approaches to therapy in regenerative medicine. In recent years, techniques have been developed to turn differentiated cells back into their original pluripotent state, or to "reprogram" them. They can then be turned into other kinds of cells again practically as desired. This new method offers enormous opportunities for stem cell research and for patient-specific and regenerative medicine. It also circumvents the ethical problems surrounding the acquisition of embryonic stem cells. At present, however, somatic cell reprogramming is still a long way from extensive commercial or clinical use because many technical and biological hurdles remain to be cleared. Within the scope of a collaborative interdisciplinary project, we are conducting research into new, substance-based possibilities of making improved reprogramming possible.

Reprogramming body cells delivers decisive benefits

Induced pluripotent stem cells (iPS cells), as reprogrammed cells are known, provide invaluable potential for patient-specific medicine. They enable the effectiveness of a drug for a certain patient to be tested on a tiny skin tissue sample that has been reprogrammed into liver tissue, for example. The skin cells can be reprogrammed into iPS cells and then turned into hepatocytes or liver cells. How these artificial hepatocytes react to the drug can then be tested. This technology also has enormous potential for regenerative medicine. In future, for example, patients will be able to donate their own cells to be turned into tissue of other cell types.

In addition to these medical prospects, iPS cells can already circumvent many of the ethical problems that are associated with embryonic stem cells.

Targeted reprogramming by means of genetic manipulation was first undertaken in 2006 (Takahashi, K. and Yamanaka, S., 2006). Somatic cell reprogramming (SCR) methods have since been modified on many occasions, and SCR can now be undertaken in many and varied ways. With a few exceptions, however, all cell reprogramming is based on the overexpression of one or more transcription factors, or genes that control the expression of a large number of other genes. In this context, frequent use is made of the so-called Yamanaka cocktail, which is made of the four genes (Oct4, Sox2, Klf4 and cMyc), that are artificially overexpressed in parallel. These genes form part of a self-regulating network, the activity of which prevents cell differentiation and makes unhindered cell division possible.

Problems due to viral integration

Introducing genes that then integrate into the cells' genetic material leaves an undesirable genomic signature, which increases the risk of cancer and makes the therapeutic application of today's methods too hazardous to the patient's health.

Another approach is reprogramming with the assistance of chemical substances. If the above-mentioned methods are combined with known small molecules to improve their efficiency, successful reprogramming can be accomplished faster. For example, Valproic acid (VPA) is the best known small molecule that can boost efficiency. Based on this observation, our project involves looking for other small molecules to take reprogramming forward or even to replace gene integration.

We were able to provide experimental evidence that combining the cAMP analogue 8-Br-cAMP with VPA increases the efficiency of Yamanaka reprogramming and that this effect is caused in part by temporarily suppressing the p53 signalling pathway (Wang, Y. and Adjaye, J., 2010).

Group photo (from left to right): Mei-Chih Liao, Ying Wang, Sheraz Gul, Nancy Mah, Jochen Supper, Miguel Andrade, Phil Gribbon, James Adjaye, Edda Klipp, Alexander Hahn, Frank Wenke, Stefan Wölfl, Axel Göhring and Ralf Mrowka (Photo: Ralf Mrowka).

Systematic improvement by means of targeted experiments

However promising iPS technology may be, this method will be unable to deliver on many of its promises unless fast, complete, signature-free reprogramming is made possible. Reprogramming with the aid of small molecules can only be achieved by a better understanding of processes within the cell during normal differentiation and induced de-differentiation. That is why we are combining different experimental high-throughput methods with mathematical modelling in this project in order to fit the individual findings into an overall picture (Fig. 1).

Before embarking on the experimental work we undertook a bioinformatic meta-analysis of gene expression profiles from reference literature measured before and after reprogramming. This analysis showed that mesenchymal-epithelial transition (MET) promotes the pluripotency of cells (Wang, Y. *et al.*, 2010). MET is the reversal of a process that occurs in embryo development (Fig. 2).

Expression profiles of somatic cells at different early stages in Yamanaka reprogramming process were then compared with the profiles of finished iPS cells and embryonic stem cells (Fig. 3). These experiments showed that viral induction and the associated immune response greatly impair the efficiency of reprogramming (Mah, N. *et al.*, 2011).

At the same time, a library of small molecules was screened for activation of pluripotency genes in order to identify candidate molecules. In parallel, we developed gene networks and dynamic models of differentiation and reprogramming (Kiełbasa, S. M. *et al.*, 2010) in order to link the experiments with existing findings (Fig. 3B).

Figure 1: Organisational diagram of the course of the project workflow

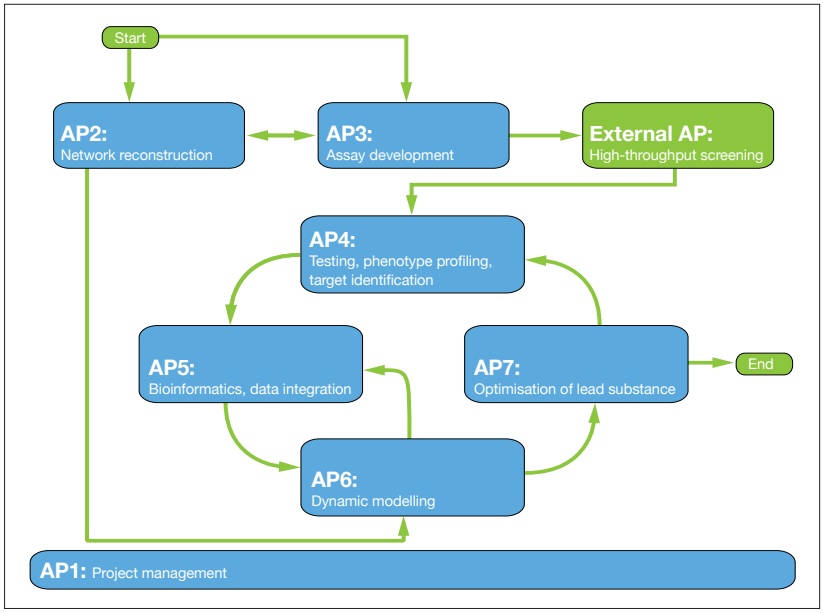

Chart: Ralf Mrowka

The different work packages (APs) run in parallel and supply themselves with data at the same time, starting with network reconstruction from literature references and the development of assays for screening. Followed by a closely knit cycle of experiment and theory that improves the results with each iteration.

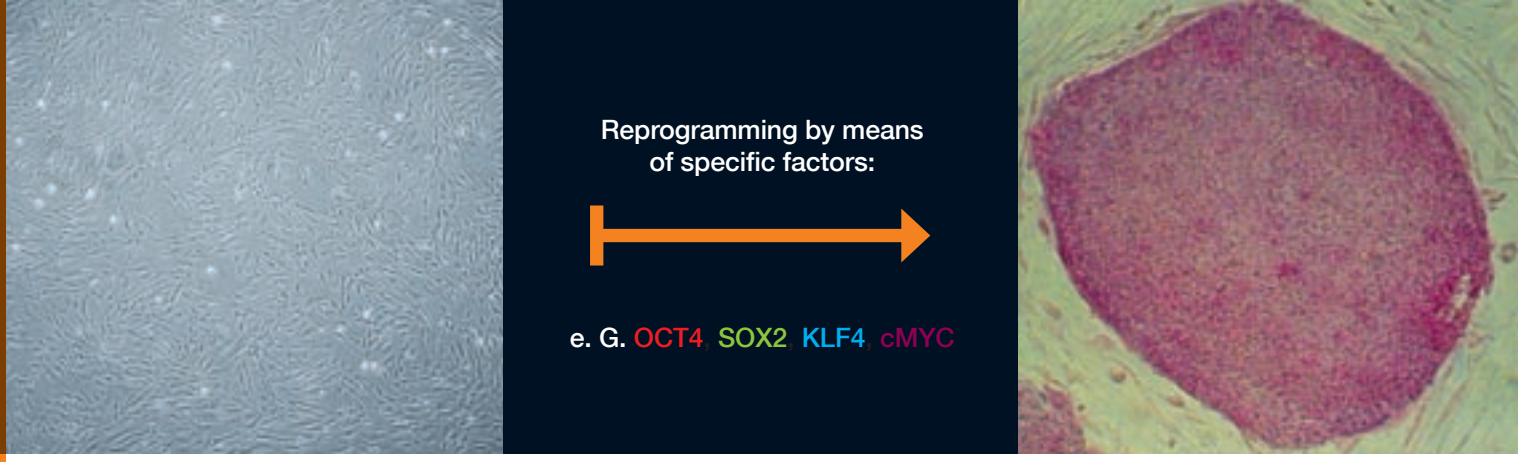

Figure 2: Microscopic images of the reprogramming of dermal fibroblasts
Skin fibroblasts (left) are infected with a combination of retroviruses that introduce the genes OCT4, SOX2, KLF4 and cMYC. Forced expression of these factors induces the reprogramming process in the skin cells during which they change their genetic expression pattern and morphology (Image: Katharina Drews).

The next step now deals with the verification and integration of the different results into a coherent model depicting the function of the molecules discovered in the chemical screen.

The needle in the haystack

Searching for small molecules that influence our gene network in the desired manner is like looking for a needle in a haystack. An incredibly large number of substances might, in theory, be suitable for reprogramming, and all need testing to see how effective they are.

To screen for candidate substances, reporter systems first had to be established to activate the stem cell genes. To this end, four luciferase reporter cell lines were specially established, each of which makes a gene detectable by means of a luminescent reaction. Luciferase reporters combine the promoter of a gene that conveys pluripotency with the gene of the luciferase enzyme, which triggers a distinct luminescent reaction that is very easy to detect upon addition of luciferin (Fig. 4). For screening, all 250,000 substances were applied individually to all four reporter lines and the corresponding luminescent reactions were measured.

The molecules that scored the most hits in screening are currently being tested for effectiveness in further experiments, with the main focus on the dynamics of gene activation. Luciferase reporters provide high-resolution data of the cellular response to the individual candidate substances and their combination.

Figure 3:

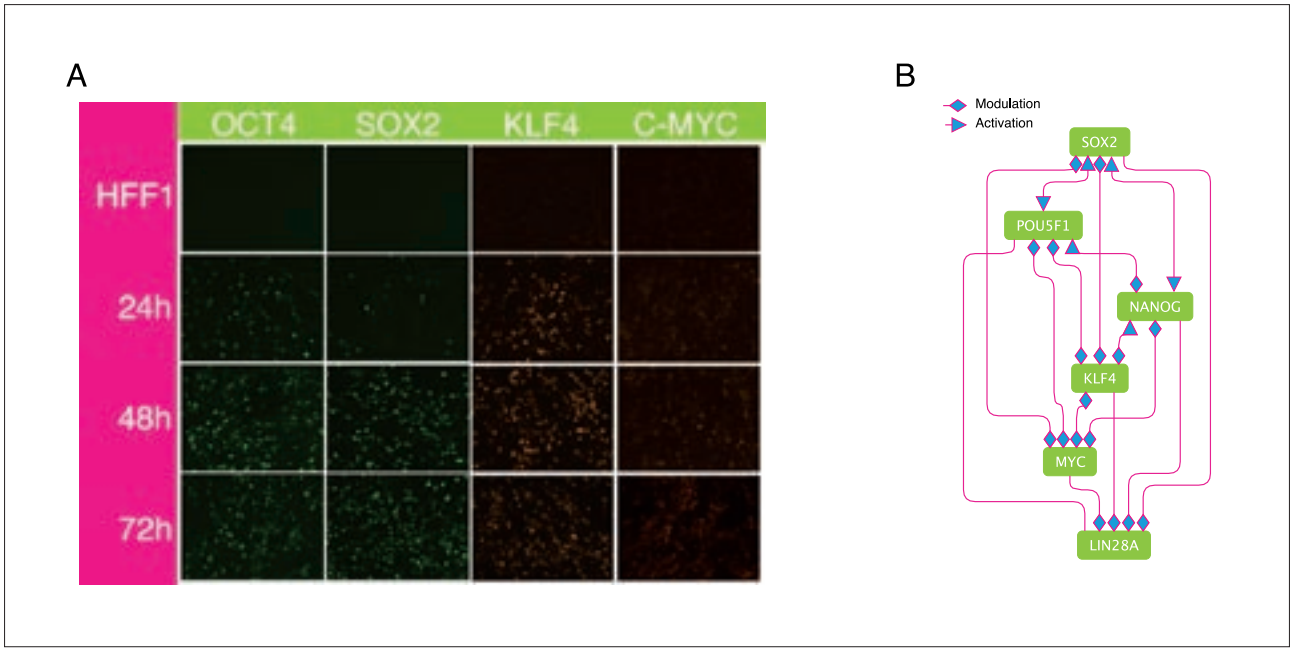

Chart: Ying Wang, Max Flöttmann

(A) Fluorescent images of the reprogramming genes during the first 72 hours of reprogramming. A DNA expression profile of the cells was made at each of these times.

(B) A network of the most important pluripotency genes identified by data mining during reprogramming.

Modelling the processes involved

Mechanistic findings about the reprogramming process are at present very limited and no comprehensive models exist that describe these findings adequately. The role of individual genes that are overexpressed during reprogramming is also still unclear.

The available data presents difficulties in mathematical modelling. Literature on the subject is plentiful but in part very contradictory. So the modellers' first task was to take stock of the existing literature and compile a comprehensive network for future use in mathematical models of reprogramming. For this task, the Genomatix company developed the GePS software application, which underwent further development in the course of the project and became an extremely useful tool.

Network modelling consisted initially of a Boolean simulation (which assigns the value "expressed" or "not expressed" to each gene and updates it in accordance with logical relationships within the network) and a comparison with data from the expression measurements at the start of reprogramming. The experiments now under way, however, will provide sufficient data for much more detailed dynamic modelling of gene regulation.

Figure 4:

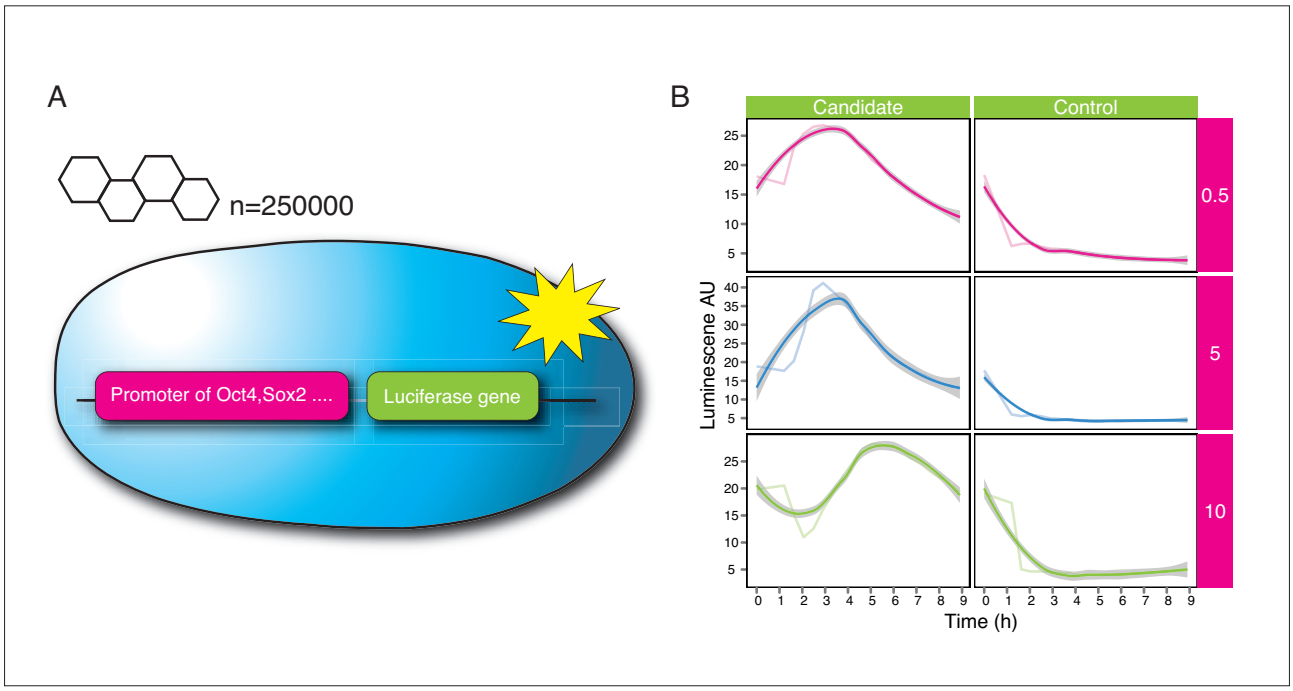

Chart: Ralf Mrowka, Max Flöttmann

(A) How the luciferase reporter system works: The promoter of the gene that is to be detected is inserted before the gene that codes the luciferase. This construct is integrated into the reporter cells' genome. In this way the promoter's activity can be established via the strength of the luciferase light reaction. Using this method, 250,000 substances were tested for their influence on gene expression.

(B) Time series of luciferase measurements in Oct4 reporter cells. The cells were treated with different concentrations (µM) of one of the substances identified in screening.

A systematic improvement in reprogramming methods can only be achieved via a better understanding of the dynamic processes during reprogramming and by identifying the biggest obstacles encountered in the process. Exact analysis of processes in the most important gene networks of differentiation and reprogramming gives us an insight into the dynamics of these processes, and by modelling them we are able to predict improvements in the reprogramming process.

The research project in brief:

Project name: drug-iPS

The drug-iPS project involves a consortium of seven German working groups and an external industry partner and is funded by the German Federal Ministry of Education and Research (BMBF) as part of its three-year Medical Systems Biology – MedSys funding programme. Research is conducted into the influence of small molecules on the reprogramming of somatic cells into induced pluripotent stem cells (iPS cells) with the aim of developing new approaches to treatment.

Partners involved (PI):

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Max Delbrück Center for Molecular Medicine, Berlin,
Miguel A. Andrade-Navarro;
Humboldt University of Berlin, Theoretical Biophysics,
Edda Klipp;
Max Planck Institute for Molecular Genetics, Molecular Embryology,
James Adjaye;
Heidelberg University, Institute of Pharmacy and Molecular Biotechnology,
Stefan Wölfl;
Genomatix Software GmbH, Munich,
Alexander Hahn.

Screening Partner:

European Screening Port GmbH, Hamburg, ESP, (Phil Gribbon and Sheraz Gul)

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medical bioinformatics against the hepatitis C virus

Novel approaches and software in systems medicine to combat viral infection

by Mario Albrecht, Hagen Blankenburg, Nadezhda T. Doncheva and Sven-Eric Schelhorn

More than a quarter of a billion people worldwide carry the hepatitis C virus, although most of them are unaware of it. This viral infection manifests itself through chronic inflammation of and considerable damage to the liver. Drug therapies applied until now are only effective in one out of two patients and have numerous side effects. Therefore, scientists intensively seek new target molecules and drugs for better therapeutic approaches. Medical bioinformatics supports this research with new methodological approaches of systems medicine and suitable software for analysing and visualising the large volumes of data generated in the lab.

A widespread, but hidden infectious disease

Hepatitis C is an inflammatory liver disease, which occurs worldwide and is triggered by infection with the hepatitis C virus (HCV) discovered first in 1989. Initially, the disease is inconspicuous in the human body, but, in the course of years, it causes the progressive destruction of the liver, which, if untreated, often leads to liver cancer and ultimately to the patient's death. The virus is transmitted between people primarily by blood and blood products, though the path of infection cannot be traced in about 30% of those infected.

HCV, like the human immunodeficiency virus (HIV) and many other viruses, is highly adaptable and changes its genome sequence continuously. This is why no vaccine against HCV exists yet and why antiviral drugs become ineffective rapidly. A further difficulty is that, for various reasons, the drugs approved until recently show an insufficient effect in half of all patients infected with HCV in Europe.

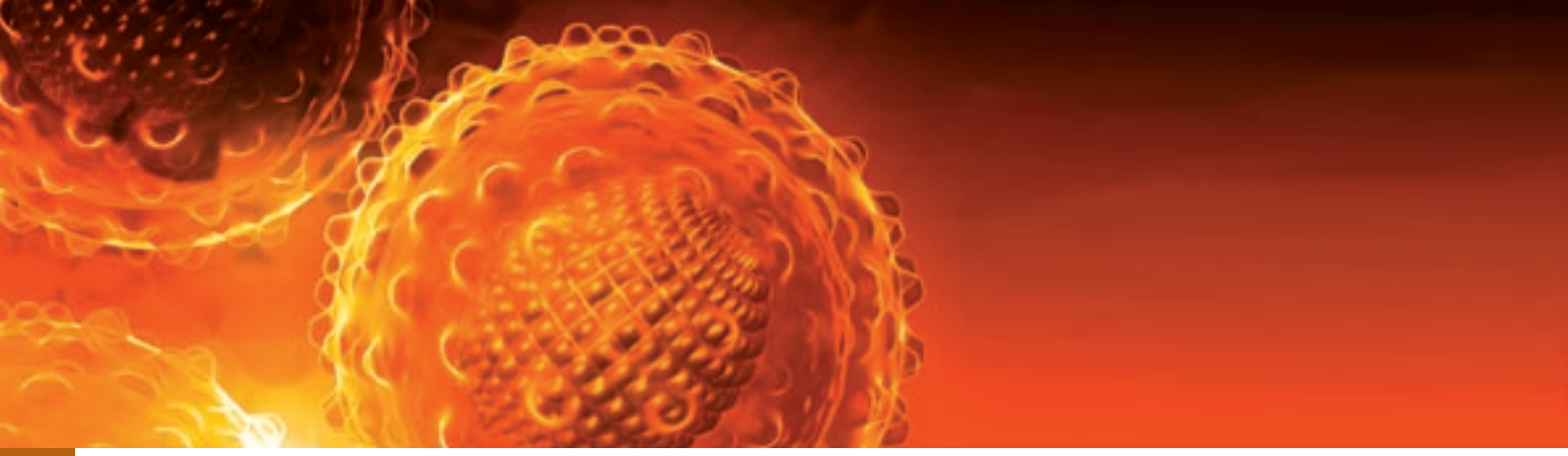
Therefore, our bioinformatics research at the Max Planck Institute for Informatics in Saarbrücken supports the worldwide search for better drugs to fight HCV. Innovative software helps to analyse viral genome variations and their impact on virus function and drug action. We also develop novel methods for the analysis of experimental data, which will be useful for discovering new target molecules of anti-HCV drugs.

Analysis of viral sequence changes

A starting point for bioinformatics support of HCV therapy is the computational analysis of the viral genome sequences found in the patient. This includes determining the sequence changes by means of which the virus responds to the latest antiviral drugs

Figure 1: The web service geno2pheno[hcv]

Image of the web service geno2pheno[hcv] for planning the therapy of HCV-infected patients. The identification of viral sequence changes requires different analysis steps of the virus genome, which are bundled in this web service (Image: Sven-Eric Schelhorn).



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and prevents them from binding directly to viral molecules and interrupting its reproductive cycle. To this end, in cooperation with the University Hospital Frankfurt and other hospitals, the viral sequences are determined at different time points of the drug therapy.

Since the latest generation of sequencing technology generates very large amounts of fragmented sequencing data, millions of short sequence fragments first have to be assigned to the right location in the viral genome. This challenging task can only be accomplished by using powerful computers and by developing and applying appropriate computational methods. Subsequently, statistical methods are used in order to identify those changes in the viral genome that make the hepatitis C virus resistant to antiviral drugs.

The resistance information provides doctors with important clues on the course of the viral infection and assists pharmaceutical researchers in finding patient-specific therapies and more effective drugs. For planning these personalised therapies, our research group develops the web service geno2pheno[hcv] (Fig. 1). The web service enables doctors to examine, quickly and free of charge, viral sequences in patients for sequence changes that confer drug resistance. This helps combating the virus more effectively and with fewer side effects for the patient.

Analysis of viral protein structures

Variations in the genome of the hepatitis C virus frequently cause changes of the spatial structures of viral protein molecules. Such three-dimensional structures and the drugs that bind to them have already been determined experimentally with single-atom resolution using X-ray crystallography. Thus, the development of new therapies can be supported by the accurate analysis of the HCV protein structures and the drugs that interact with them. For this purpose, structural biologists and pharmacologists often use specialized software programs for the spatial visualisation of viral protein structures that may consist of up to several thousand atoms. This provides important insights into the molecular mechanisms of the viral protein structure and its function, which might be influenced by drug binding and sequence changes. For example, the latest antiviral drugs block

Figure 2: Visualisation and analysis of the protein structure of the HCV protease NS3-4A using RINalyzer

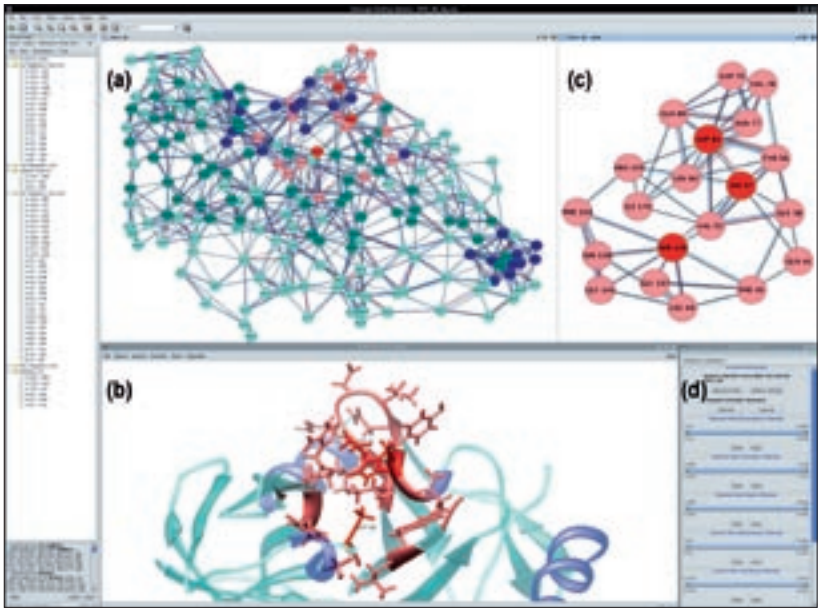

Image: Nadezhda T. Doncheva

(a) Structure network of the HCV protease in 2D;
(b) Protein structure of the protease in 3D;
(c) Close-up showing functionally relevant interactions in the active site of the HCV protease;
(d) Slide control for the visual analysis of the structure network.

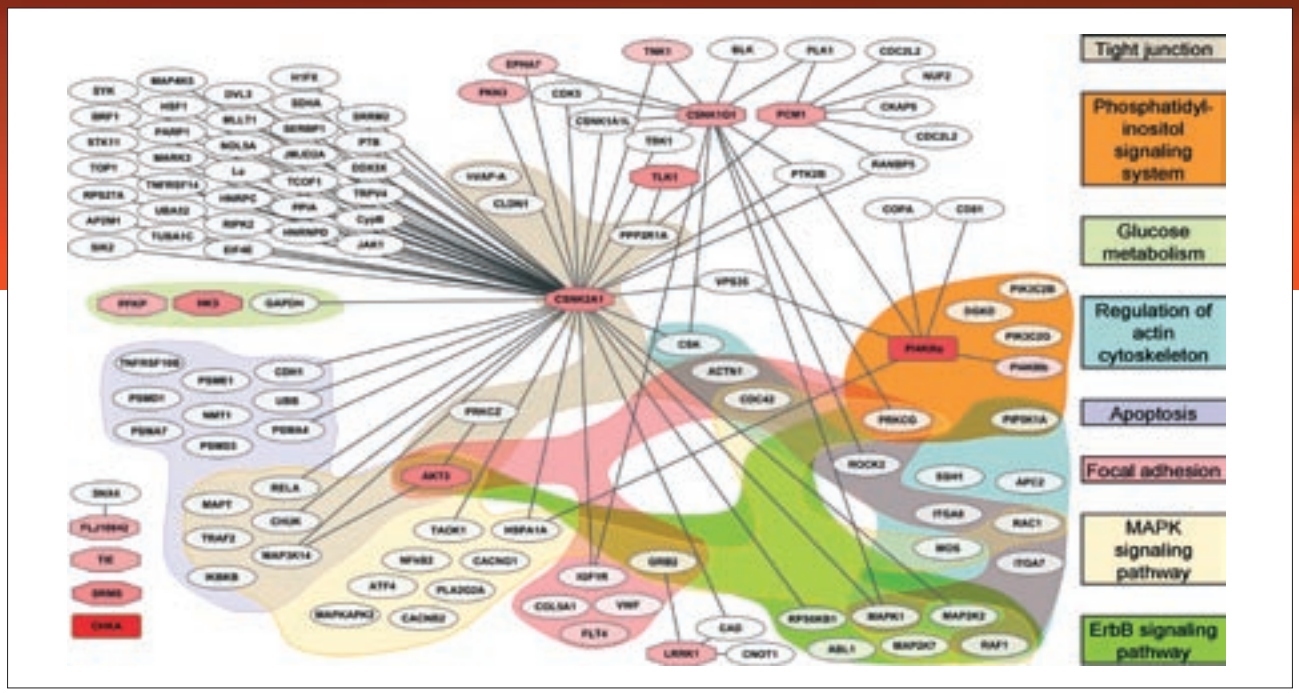

Figure 3: Schematic drawing of selected human host factors of HCV and their interactions in different molecular processes of the human cell

The individual host factors are depicted as ovals, octagons, or rectangles, depending on whether they have been previously known host factors, newly found in the lab during the present study, or already known from earlier experiments, respectively. The deeper the red shade of a host factor, the greater its importance in the experiment for the viral life cycle in the human cell. Interactions between host factors are shown as connecting lines, with some signalling pathways in which certain host factors are involved are highlighted in colour (Image: Hagen Blankenburg).

the function of the viral protease NS3-4A, which is vital for HCV reproduction, whereupon the virus responds by changing its sequence and structure (Welsch *et al.*, 2008).

To further simplify the structural analysis of proteins, in view of the large number of atoms to be taken into account, our research group develops novel integrative visualisation software (Doncheva *et al.*, 2011, 2012). It visualises the atoms of protein structures and their interactions as network in only two dimensions in addition to three dimensions as before (Fig. 2). This simplified network representation complements the established three-dimensional structure analysis, particularly when studying numerous complex interactions between atoms in the protein structure. Because of the great number of interactions, especially across large molecular distances, they can no longer be displayed clearly in three dimensions on a two-dimensional computer screen. This makes our new approach especially suitable for identifying all sequence variations that are relevant to drug effectiveness as well as for visualising and detailed understanding their molecular effects on protein structure and function.

Analysis of human host factors

Drugs that do not target viral molecules directly, but act indirectly by targeting molecular factors in the human host that are essential for the virus, offer an alternative approach to combating HCV. Viruses need these human host factors in order to enter liver cells, to replicate in them, ultimately to leave them and to infect further cells. Better knowledge of the many host factors therefore enables a more comprehensive understanding of the different stages of the viral life cycle in human cells. The most important factors are then potential human target molecules of antiviral drugs, because HCV cannot inhibit the effectiveness of this kind of drug therapy by changing its viral genome.

To this end, our research group cooperates with virologists at the University Hospital Heidelberg in their efforts to discover new human host factors for HCV. The lab experiments performed for this purpose generate extensive measurements that require special bioinformatics methods for data analysis and interpretation. A main focus of our work is to enrich these experimental findings with additional function information from other molecular bio-

logy data sources in order to interpret the individual lab results in the context of a more global, cellular network of interacting molecules (Fig. 3). Amongst other things, this facilitates the identification of relevant host factors and allowed us to explain the molecular mechanisms in which HCV uses a human protein, the lipid kinase PI4KIIIα, for viral replication (Reiss *et al.*, 2011). Thus, impairing the protein function of this critical host factor is now a possible target of future drugs to block the viral life cycle.

In summary, medical bioinformatics contributes, through integrative data analysis, towards uncovering host factors and their molecular interactions as potential targets of new drugs in human cells. Computational methods speed up the elucidation of disease causes at the molecular level and enable the faster development of drugs.

The research project in brief:

Dr. Mario Albrecht has been research group leader for Molecular Networks in Medical Bioinformatics at the Max Planck Institute for Informatics and in the Multimodal Computing and Interaction (MMCI) cluster of excellence in Saarbrücken. He has also been a member of the clinical research group "Mechanisms of resistance development and optimisation of antiviral strategies in hepatitis C virus infection using integrative models of biomathematics and bioinformatics" (KFO 129) funded by the German Research Foundation (DFG). Recently, he has become Professor of Bioinformatics at the Institute of Biometrics and Medical Informatics at the University Medicine Greifswald.

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center systems biology (CSB)

at the University of Stuttgart

by Matthias Reuss

The Center Systems Biology (CSB) at the University of Stuttgart was established in 2005, making it one of the first cross-faculty systems biology centres in Germany. A distinctive aspect of systems biology research in Stuttgart is the close networking of systems, engineering and life sciences as well as its cross-university positioning.

This close and successful collaboration between biology and engineering and systems science dates back to the "bioprocess engineering" focus project, carried out from 1988 to 2000 and funded by the German Federal Ministry of Education and Research (BMBF), in which early research in the main areas of systems biology was conducted. Further strengthening of the systems approach to biology followed in the early 1990s with consistent developments in the new research discipline of "metabolic engineering" and, from the mid-1990s, with the research program "biosystems engineering" funded by the state Baden-Württemberg. In the course of the further development of this concept, the CSB was established in 2005. The University of Stuttgart's Center is unique in closely linking biological sciences, engineering and systems science.

Work at the Center began in 2006 with the CSB Research Program, 12 projects in which working groups from six University of Stuttgart faculties and the University of Tübingen's Proteome Center were jointly involved. The federal state of Baden-Württemberg provided initial three years of funding from its "Zukunftsoffensive III" futures initiative to help develop the CSB. The sustainability of this funding was demonstrated in the years that followed by the successful raising of EUR 17 million towards collaborative research in systems biology.

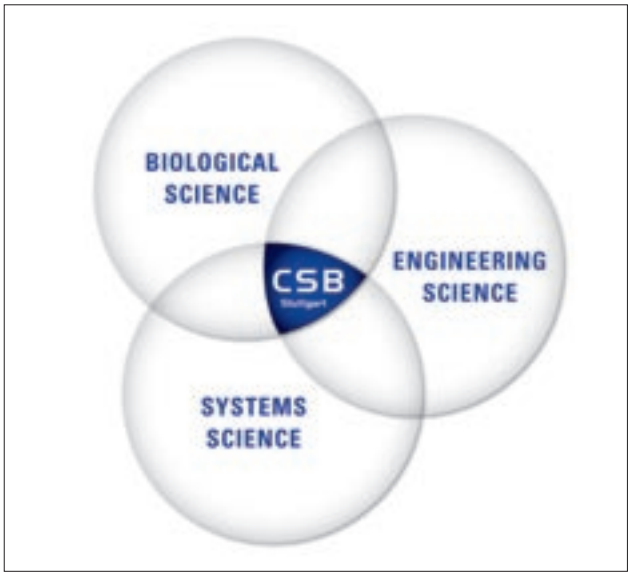
Task and structure of the CSB

The task of the Center, which is independent of university faculties, is to coordinate cross-faculty research projects in the field of systems biology and to provide a platform for the participating institutes and faculties. The University of Stuttgart provides the infrastructure required.

A central coordination unit draws up joint funding applications, and project planning and administration converge here.

Another part of the Center's infrastructure is the Central Laboratory for Microscopy and Image Analysis at the Institute for Cell Biology and Immunology, which places modern microscopic equipment and additional services at CSB members' disposal.

Disciplines involved



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SYSTEMS SCIENCE

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Institute for Systems Theory and Automatic Control (IST)

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Research fields

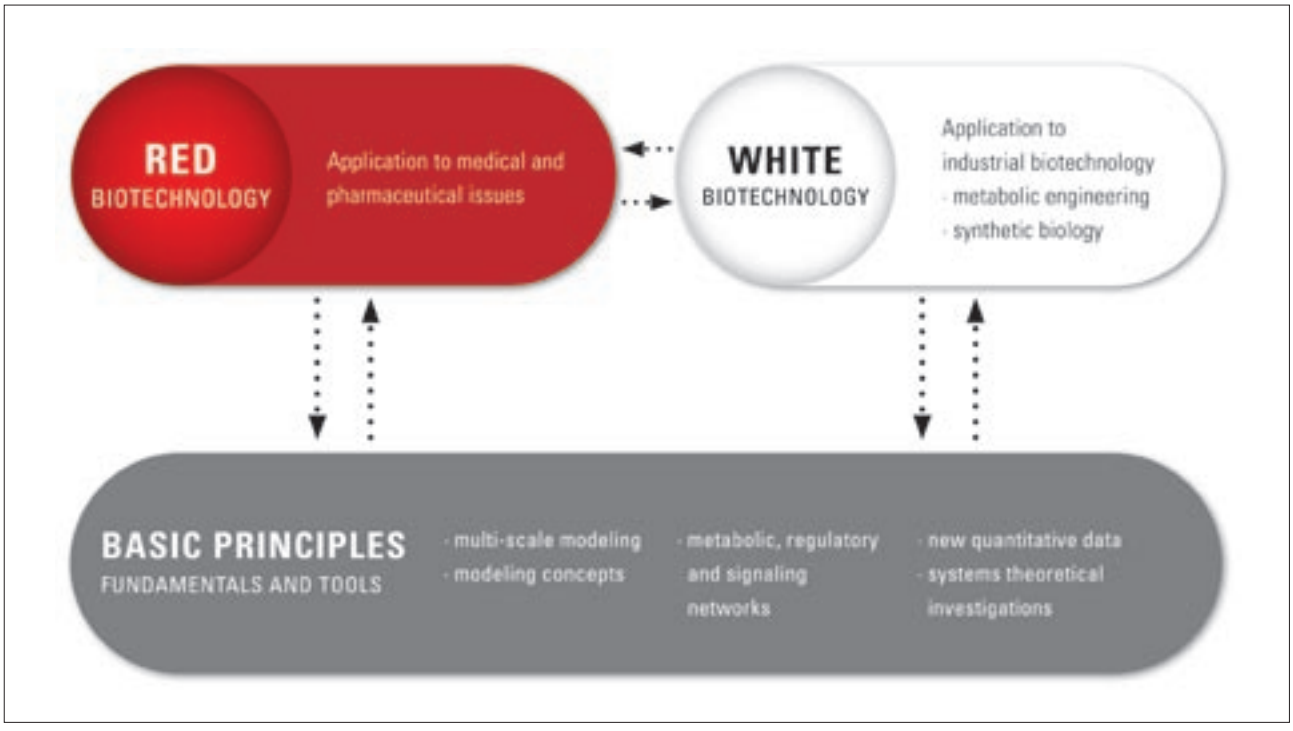
Research in systems biology at the Center is divided into three areas: The development of methods and tools and research in red and white biotechnology (Fig. 1).

The special combination of the research fields development of methods and tools as well as applications in white and red biotechnology provides outstanding opportunities to integrate into systems biology previously fragmented expertise on the dynamic behaviour of networks such as metabolism, regulation and signal transduction.

Another focal point of research at the CSB is multi-scale modelling and simulation. The effects of interaction between the different levels in biomedical applications (molecules, cell networks, cells, tissue, organ and organism) or in industrial production (molecules, cell networks, population, bioreactor and large-scale production plant) are manifold and complex (Figs. 2 and 3). Novel modelling and simulation concepts are required to solve these problems.

An example of an issue arising from the area of red biotechnology is the research project "FORSYS Partner: A Systems Biology Approach towards Predictive Cancer Therapy". As part of this project, a model-based systems biology approach is taken at the CSB to develop new active agents and methods for treating cancer. The main focus is on delivery of the active agent to the target.

Figure 1: Focal research activities at the CSB



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Engineering (IBVT)

Other medical-pharmaceutical issues are dealt with at the CSB in projects such as New Methods in Systems Biology – SysTec and Medical Systems Biology – MedSys.

The University of Stuttgart's technical background has made its mark on research in the life sciences, and a distinctive aspect of the CSB is sure to be the significant contribution of engineering towards research into systems biology. This leads on to a further aspect: The Center's focus on white biotechnology, an area in which research findings from systems biology are used to design microorganisms for technical production processes, for example. Research in Stuttgart includes work on the metabolic processes

Figure 2: Multi-scale modelling in red biotechnology

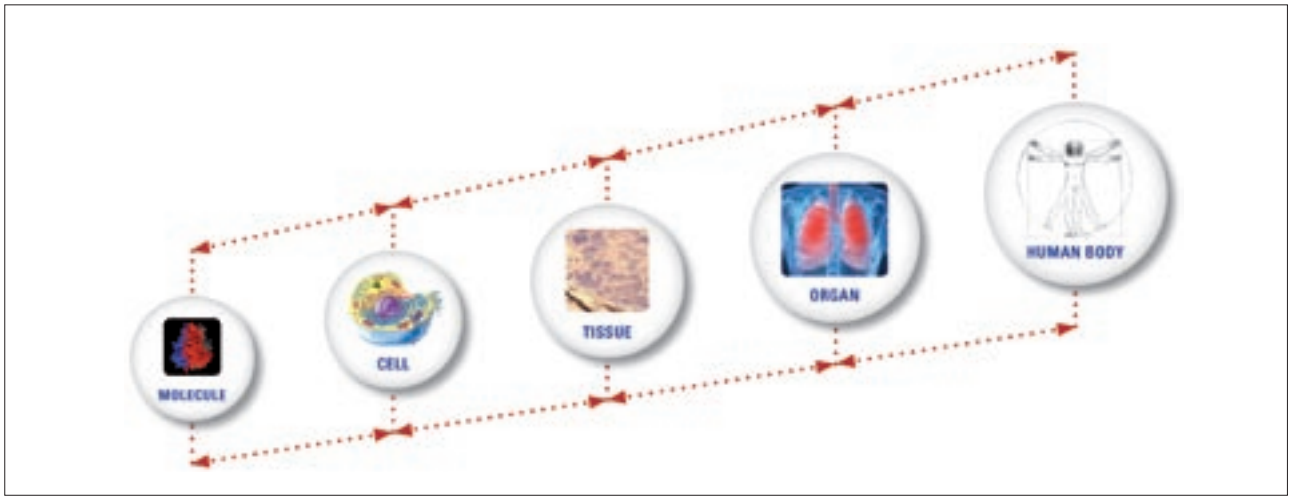

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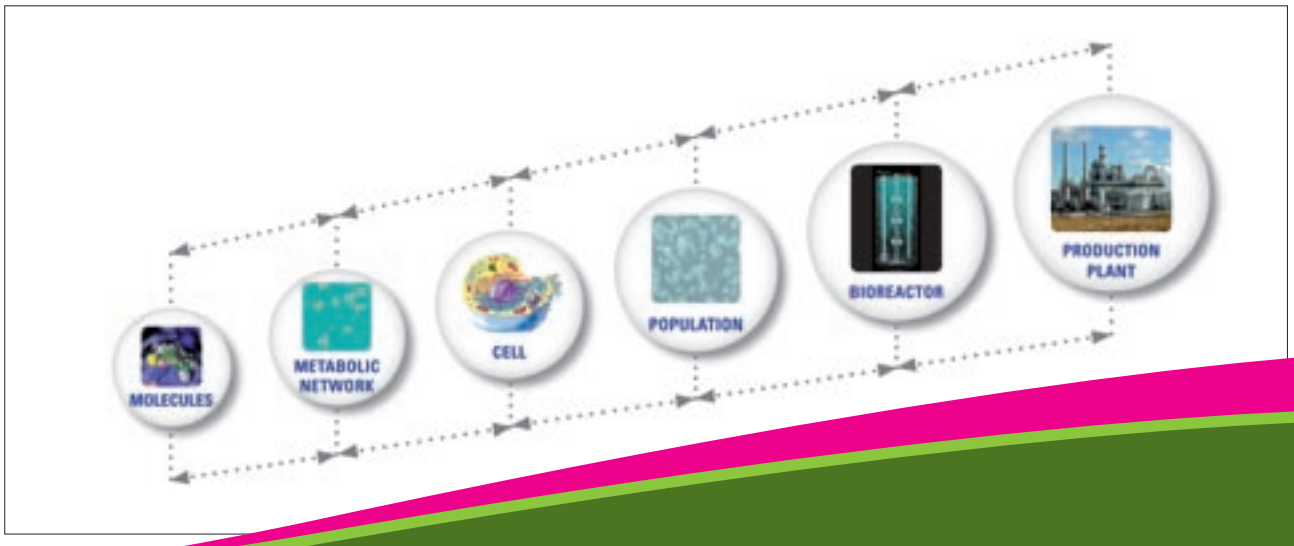
Figure 3: Multi-scale modelling in white biotechnology



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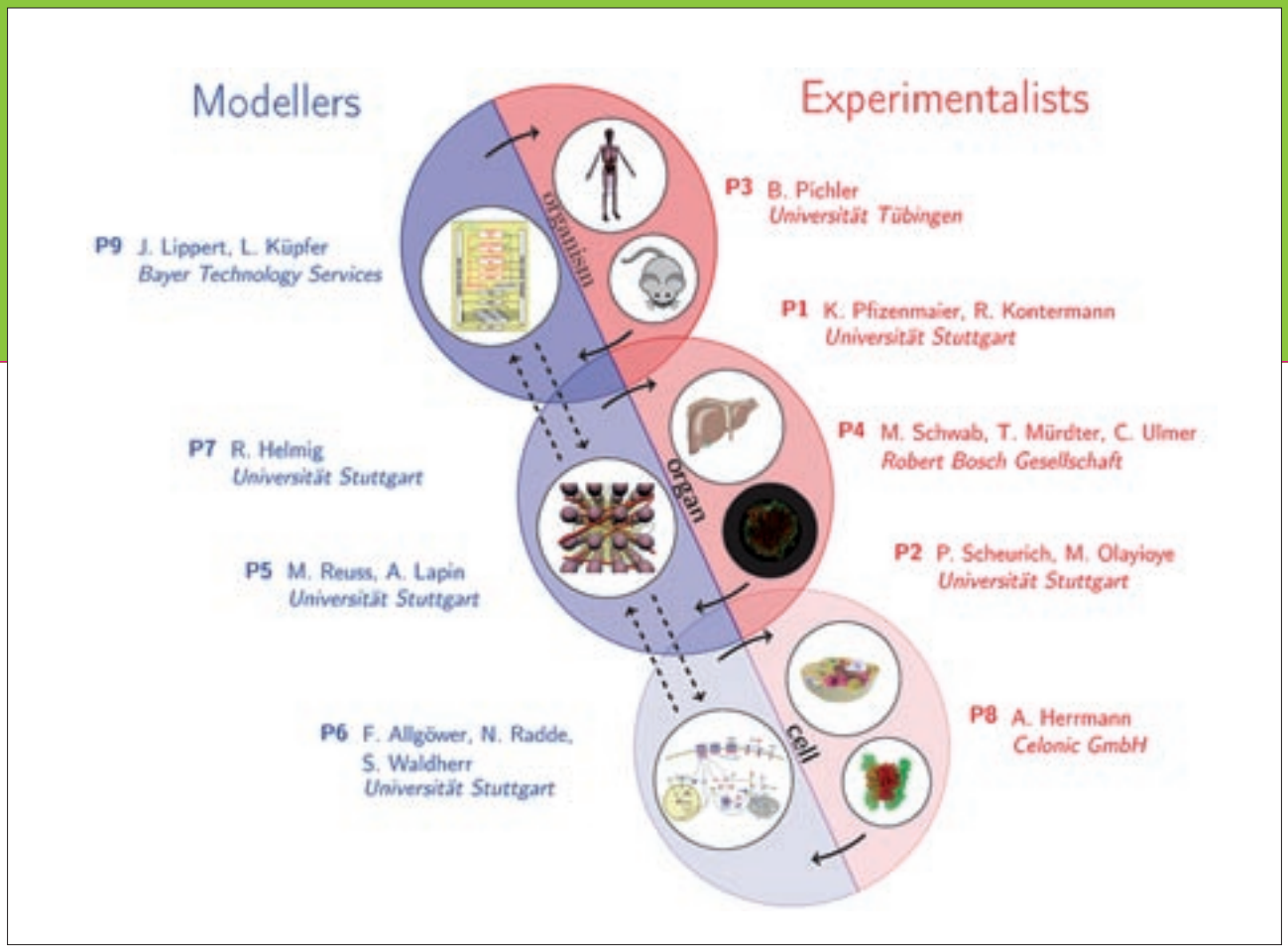

Chart: Jan Hasenauer, IST, University of Stuttgart

Figure 4: Example of multi-scale modelling approaches in red biotechnology: The PREDICT Project

of *Pseudomonas sp., Escherichia coli, Corynebacterium sp., Saccharomyces cerevisiae*, etc. with a view to optimising industrial processes in the long term as well as developing new biotechnological processes.

At present, six University of Stuttgart faculties are involved in CSB research projects. Very important are also the teaching and research units directly related to systems biology with which the Center collaborates very closely. These include the Simulation Technology cluster of excellence, SFB 716 (dynamic simulation of systems with large particle numbers) and the degree courses in Technical Biology, Engineering Cybernetics, Process Technology and Simulation Technology. Plans for a Master's degree programme in systems biology have also been completed.

The Center also has external partners at the University of Tübingen, the University of Hohenheim and the University of Magdeburg. External CSB partners include non-university institutions and industrial research partners.

For further details, visit the Website at:
www.centersysbio.uni-stuttgart.de

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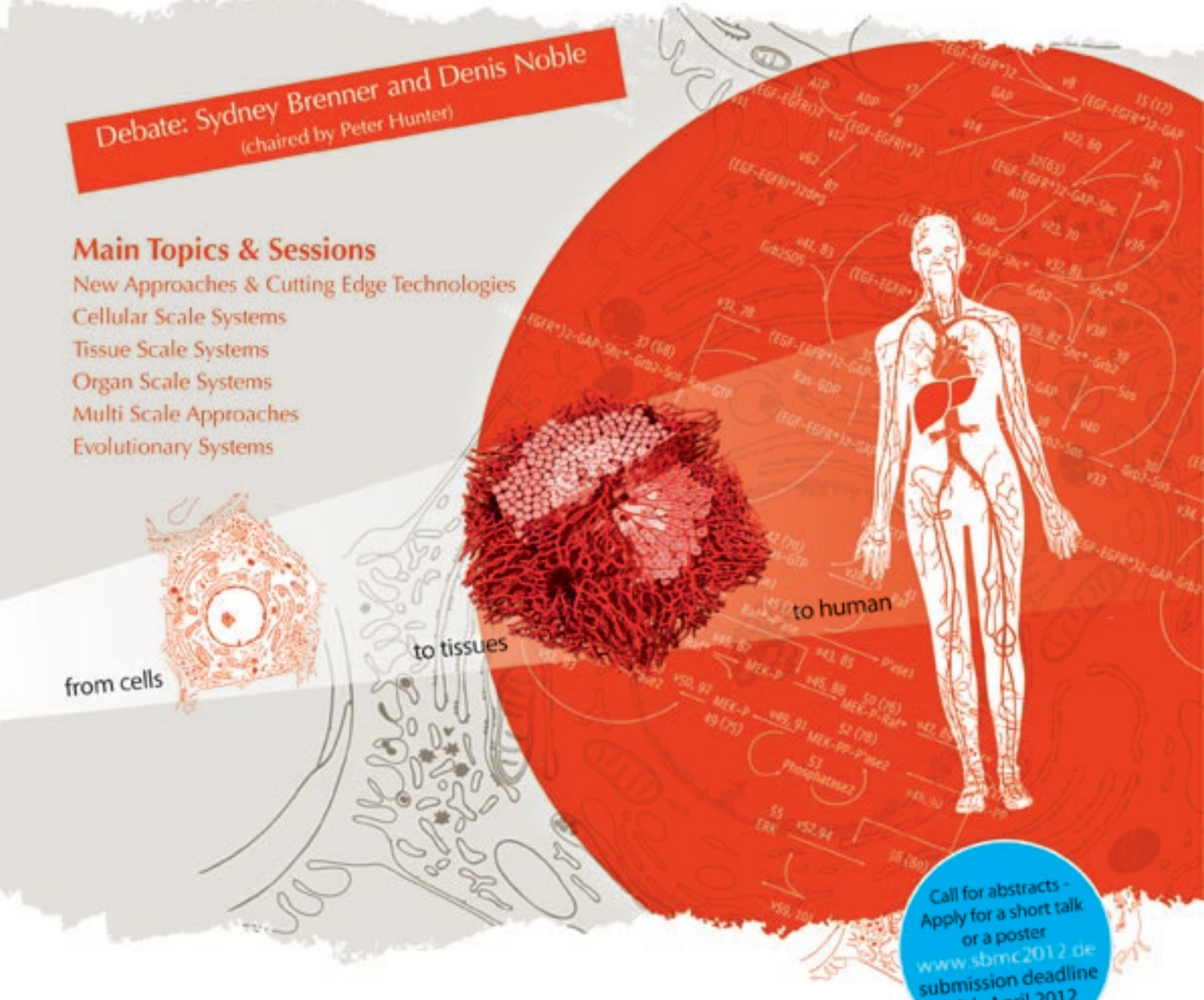

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organised by
virtual liver network

catabolic network dynamics in environmental bacteria

Quantitative mass spectrometry deciphers adaptation to changing environmental conditions

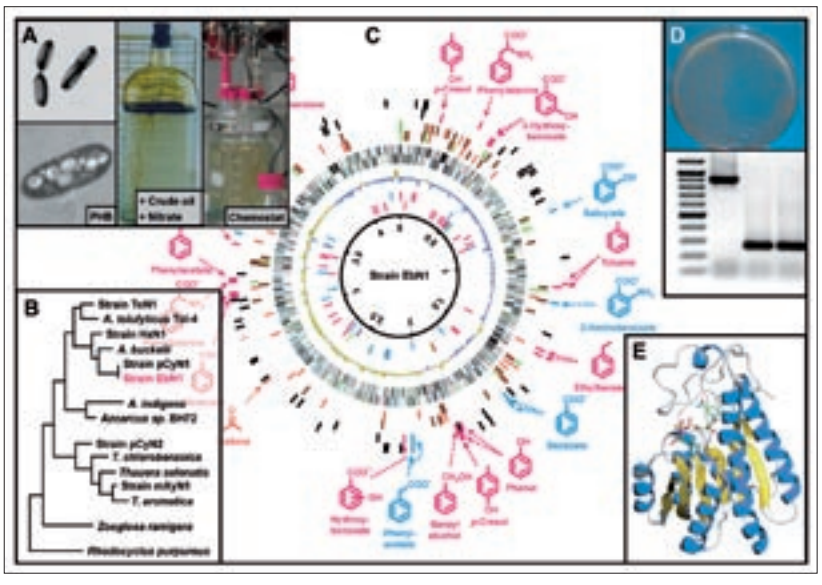
by Thomas M. Halder and Ralf Rabus

Microorganisms exhibit the most versatile and powerful metabolism of all creatures. Therefore, they are the driving force of global and climate-relevant element cycles as well as a valuable resource of innovative biocatalysts and products for biotechnology. Application of differential proteomics (context-specific coverage all cellular proteins) enabled the discovery of novel degradation capacities and networks in environmental bacteria. An important challenge for future systems biology research with model organisms is to absolutely quantify the protein components of individual metabolic modules. Here, "selected reaction monitoring" (SRM) is a promising approach, since it allows for targeted quantification of single, known proteins in complex mixtures.

Degradation specialists as driving force of global element cycles and biotechnological resource

With approximately 4–6 × 10^{30} cells, prokaryotes represent the "unseen majority" on our planet, with a total biomass equaling that of all plants (Whitman *et al.*, 1998). Due to their manifold degradation capacities, bacteria play a central role in maintaining the global element (carbon) cycles. Anaerobic (in the absence of O_2) degradation processes are of particular relevance, since O_2-free conditions prevail in most habitats of the biosphere. Next to the glycosyl(sugar)-molecule the aromatic ring is the second most abundant organic-chemical structure in nature. For instance, aromatic compounds are the building blocks of the wood polymer lignin (30% of biologically fixed carbon) and of proteins, and represent important constituents of crude oil. Interest in the microbial degradation of aromatic compounds arises not only from concerns about the global carbon

Figure 1: "*Aromatoleum aromaticum*" EbN1 as model organism for systems biology of anaerobic degradation of aromatic compounds



A) Formation of PHB; anaerobic growth with crude oil; chemostat.

B) Simplified phylogenetic tree of strain EbN1 (red) and related anaerobic degradation specialists (aromatics, alkanes and monoterpenes).

C) The 4.7 Mb genome (Rabus *et al.*, 2005) with gene clusters for aromatic compound degradation.

D) Growth on agar plates and genetic system (modified from Wöhlbrand and Rabus 2009).

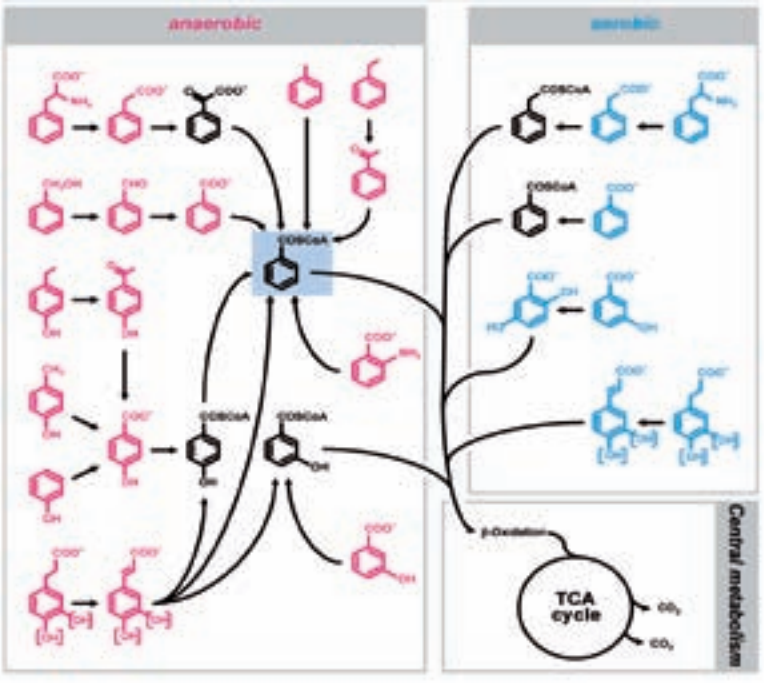
E) Cristal structure of the (S)-specific 1-phenylethanol dehydrogenase (modified from Höffken *et al.*, 2006).

budget, but also from a fundamental biochemical perspective. In contrast to the polar sugars, aromatic compounds are chemically very stable and therefore difficult to degrade. An aerobic (with O_2) life style allows microorganism to employ highly reactive oxygen-species for challenging reactions during the degradation of aromatic compounds. This is not possible in case of the evolutionarily older anaerobic (without O_2) life style, which also has to manage with less energy for metabolism. Anaerobic bacteria have evolved a multitude of intriguing biochemical reactions to anaerobically degrade aromatic compounds (Fuchs *et al.*, 2008). These reactions do not occur in standard bacteria such as *Escherichia coli* and *Bacillus* spp. and have only recently been discovered. They represent a novel, valuable and sustainable resource for diverse applications in white biotechnology, e.g. stereochemically selective reaction control or mild reaction conditions as substitute for established difficult or extensive chemical catalysis.

Proteogenomic discovery of novel catabolic networks: Entrance to systems biology

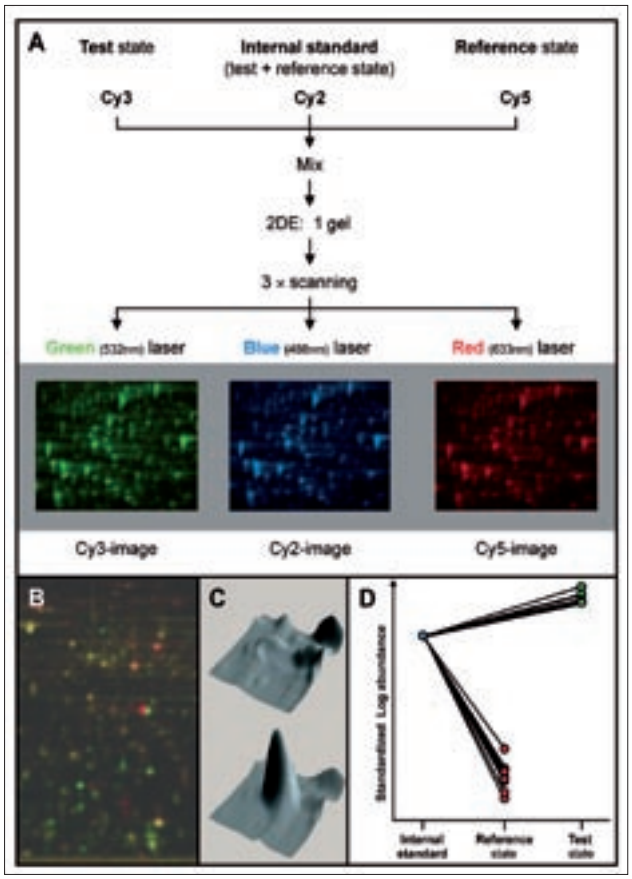
The environmental bacterium "*Aromatoleum aromaticum*" EbN1 (Fig. 1) is capable of degrading a multitude of aromatic compounds in the presence or absence of molecular oxygen (O_2). The complete genome sequence of this bacterium allowed for the first time to reconstruct (*in silico*) the catabolic (degradation) network (Fig. 2) of an anaerobic hydrocarbon degrader. To experimentally verify the predicted network, comprehensive studies with 2-dimensional difference gel electrophoresis (2D DIGE; Fig. 3) were conducted (Wöhlbrand *et al.*, 2007). These lead to the discovery of further new, genomically not predicted degradation reactions and pathways (Wöhlbrand *et al.*, 2008). In addition, the 2D DIGE studies unraveled the substrate-specific regulation of individual degradation pathways (subproteome; sets of proteins that are formed only under defined conditions) against the background of the non-regulated ("constitutive") core proteome (set of proteins that is present under all conditions). The gel-based

Figure 2: Degradation of aromatic compounds in strain EbN1



The catabolic (degradation) network of "*Aromatoleum aromaticum*" EbN1 is mainly composed of aromatic compound-specific degradation pathways (modules) (modified from Rabus *et al.*, 2005; Wöhlbrand *et al.*, 2008; Wöhlbrand and Rabus 2009; Trautwein and Rabus, unpublished). Red and blue colored aromatic compounds represent anaerobic and aerobic growth substrates, respectively. Benzoyl-CoA (boxed) is the central intermediate. Terminal oxidation (to CO_2) proceeds via the TCA-cycle.

Figure 3: Global differential proteome analysis with the gel-based 2D DIGE approach



A) Schematic representation of the working steps involved in a 2D DIGE experiment.

B) False color representation of an overlay of Cy2,3,5-images.

C) 3D-image of a differentially abundant protein spot.

D) Determination of relative differences in protein abundance.

2D DIGE approach covered the majority of soluble proteins of the catabolic network. Taken together, a first step towards a deeper understanding of "metabolic management" as survival or success strategy was achieved. Cooperation with the chemical industry allowed demonstrating the biotechnological potential (Breuer *et al.*, 2008) of a stereospecific dehydrogenase (Höffken *et al.*, 2006) from the anaerobic ethylbenzene degradation.

Absolute quantification as future challenge for systems biology

Proteins as biocatalysts represent the true actors in all cells. It is thus an obvious systems biology demand for proteomics research to accurately quantify protein abundance changes in response to environmental perturbations. To date, a relative quantification of context specific proteome signatures was achieved in environmental bacteria by combining hypothesis-driven physiological experiments with global proteome profiling (Zech *et al.*, 2011; Tebbe *et al.*, 2009). An intrinsic advantage of global approaches (e.g. 2D DIGE) is the potential to discover the formation of thus far disregarded proteins in the context of defined physiological conditions. The ultimate goal of systems biology is to quantitatively determine, predict and model all cellular processes and reactions according to space and time. In particular on the level of reactions an integration of activity and absolute abundance of enzymes will be required. Targeted, absolute quantification of selected proteins from complex mixtures and against difficult matrix background is enabled by the "selected reaction monitoring" (SRM) approach (Gerber *et al.*, 2003). To date, SRM is mainly used for monitoring few selected biomarkers across high sample numbers (Surinova *et al.*, 2011). The application of SRM in global studies for the purpose of quantitative coverage of a large set of target proteins is only at its early beginnings (Picotti *et al.*, 2009).

Contrasting the above described global quantitative approaches, SRM is a targeted method (Fig. 4), allowing for relative as well as absolute quantification of selected proteins (Gerber *et al.*, 2003); the identity of the target proteins has to be known in advance. Proteins possess defined amino acid sequence sections (peptides), which are unique for an individual protein. These so-called proteotypic peptides with known sequence and mass are used for the quantification of defined proteins by means of SRM. Initially, all protein species (~10^3–10^4) of a sample are digested into a complex peptide mixture (~10^5–10^6 different peptides) by specific enzymes. In a first mass spectrometric step, peptides having the mass of the proteotypic peptides are specifically selected by a mass filter (quadrupol). These selected peptides are then further fragmented into smaller peptides by a second quadrupol. Finally, the specific detection of defined fragment ions of the proteotypic peptide is achieved by a third quadrupol. Usually at least three such fragment ions ("transitions") are detected for each proteotypic peptide. Double mass filtering allows highly selective and sensitive determination and quantification of these transitions, also in complex samples such as whole cell lysates. Thus, all enzymes of a metabolic pathway of interest could in principle be quantified in a single SRM analysis of the cell lysate of a bacterial culture, i.e. selected known needles could be fished from a large hay stack in a targeted and efficient manner. Comparing the protein abundances of individual degradation modules across different nutrient supplies or cultivation parameters allows insights into how bacteria virtually play the piano of their metabolism to adapt to changing environmental conditions.

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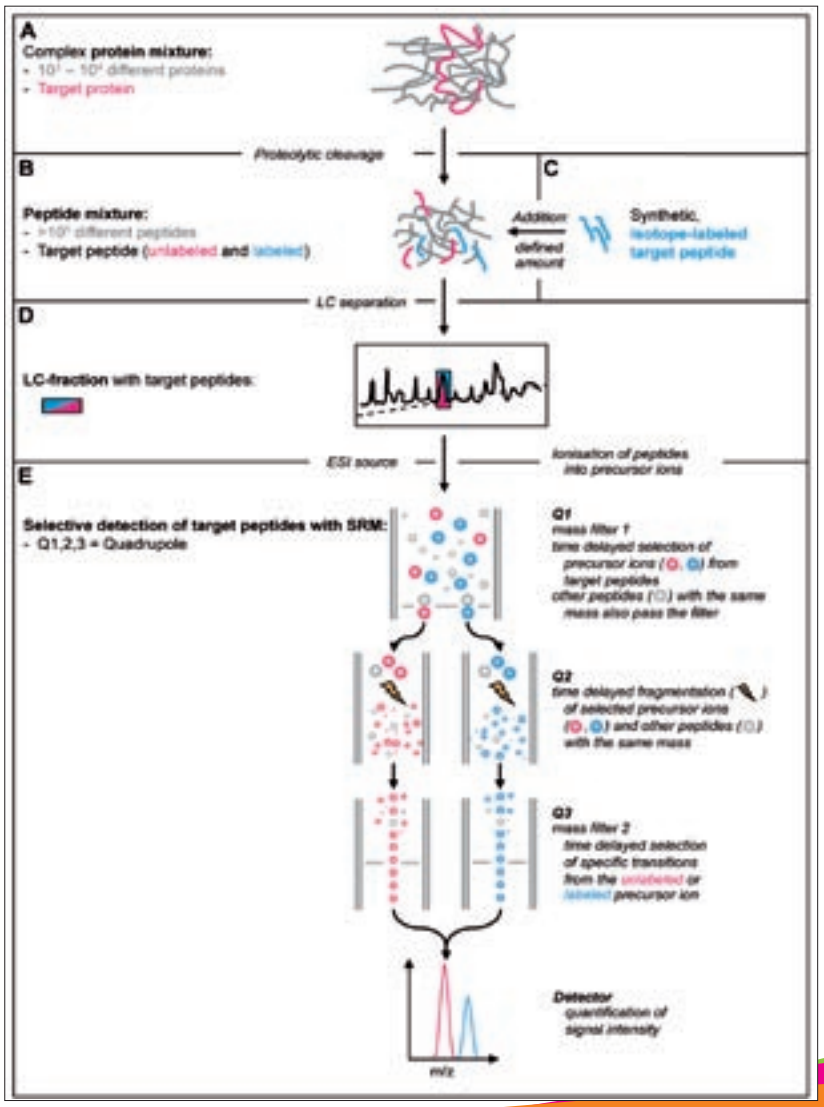
Figure 4:



General principle of absolute quantification of (proteotypic) target peptides from complex protein mixtures by means of **S**elected **R**eaction **M**onitoring (**SRM**).

population census in the cell

The first comprehensive quantification of mammalian gene expression

by Björn Schwanhäusser

Proteins are the real movers and shakers concerning how practically all vital processes function. They transport nutrients in the blood, protect us from infections and enable us to think. In view of their central role for the cell, precise control of protein concentrations is very important. If this process, also known as gene expression, becomes imbalanced, diseases such as cancer may occur. The four fundamental control variables that regulate gene expression include the transcription that occurs in the nucleus, cytoplasmic protein translation and mRNA and protein degradation. We, an interdisciplinary team at the Max Delbrück Center (MDC) and the Berlin Institute for Medical Systems Biology (BIMSB), have succeeded in accurately measuring gene expression control on a global scale for the first time. This success is based on the combination of high-precision mass spectrometry and state-of-the-art sequencing technology along with novel pulse labelling procedures. The surprising result was that the control of protein levels is exerted predominantly in the cell's cytoplasm and not, as expected, in its nucleus.

The central dogma of biology, a hypothesis proposed by Francis Crick, has, since 1958, described the multistage flow of information in cells to create proteins in accordance with the blueprints stored in our genes. Genes are first rewritten as so-called messenger RNAs (mRNAs). This process, known as transcription, takes place in the nucleus. The mRNAs leave the nucleus and serve in the cytoplasm as a matrix for protein production (translation) by the ribosomes. In recent decades research has concentrated almost exclusively on tracing faulty genes, i.e. genes associated with disease and their transcription in the nucleus. It is now clear, however, that to check protein quantities and thereby gain an understanding of diseases, processes that take place after the mRNA synthesis are also of great importance (de Sousa Abreu *et al.*, 2009). They include the degradation of mRNAs and the production of proteins as well as their degradation. In the past, these so-called post-transcriptional and post-translational processes were considered in isolation and only for individual genes. As a consequence, firm conclusions on overall control of gene expression were virtually impossible. Despite intensive research it was therefore unclear how significant the individual processes – transcription, translation and mRNA and protein stabilities – are for the control of gene expression.

Technological infrastructure as the key to success

The aim of our work was therefore to take a systems biology approach and bundle the enormous capacities and opportunities provided by the latest technologies in order to gain a comprehensive, quantitative picture of the gene expression cascade (Schwanhaeusser *et al.*, 2011). The infrastructure required was ensured through close cooperation between the Berlin Institute for Medical Systems Biology (BISMB), founded in 2008, and the Max Delbrück Center (MDC). It enabled us to generate, evaluate and compare experimental data of the proteins and mRNAs of several thousand genes and integrate them in a mathematical model that for the first time precisely reflects gene expression.

All of the experimental data was acquired with the aid of fibroblasts from mice, which were maintained in cell culture vessels. We began by measuring mRNA and protein turnover and deduced from it the cellular half-life times of mRNAs and proteins. In doing so we combined for the first time two metabolic labelling strategies that do not influence the cellular physiology (Fig. 1). For the quantitative measurement of protein turnover we used the so-called SILAC (stable isotope labelling by amino acids in cell culture) strategy based on incorporating heavy (H) amino acids (AA) in proteins (Mann, 2006). In contrast to normal, light (L) AA, heavy AA are marked by stable isotopes with a heavier molecular weight. If cells are transferred for a certain time (pulse labelling) from a culture

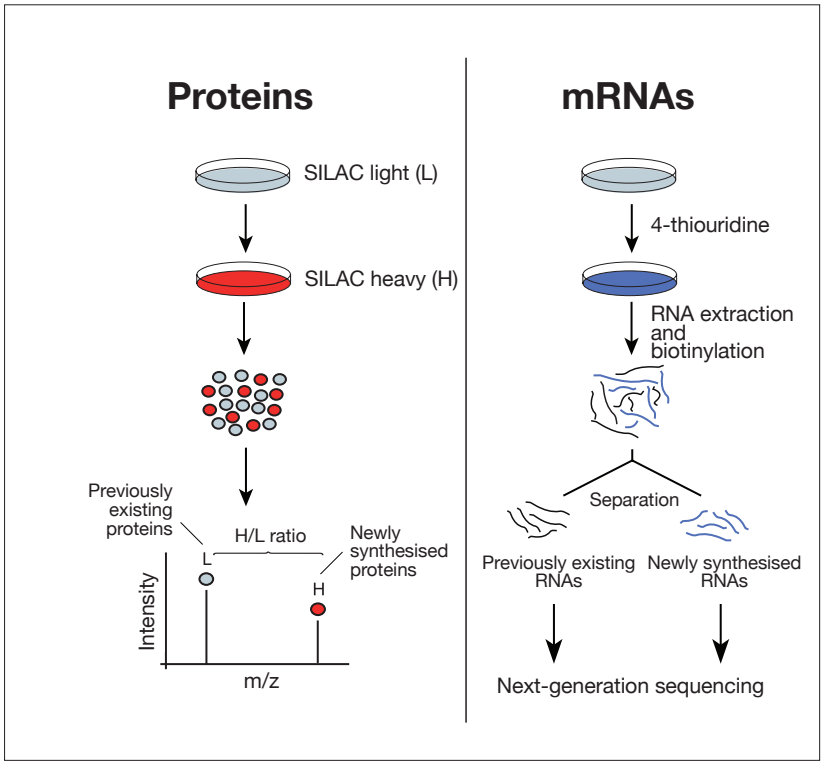
Björn Schwanhäusser (Photo: Matthias Sury).

medium that contains light AA to a medium with heavy AA, all newly synthesised proteins are created in the heavy form (Fig. 1, left). Pre-existing light proteins are, in contrast, degraded over time. A mass spectrometer can be used to distinguish between light and heavy proteins. The protein turnover rate, or H/L ratio, is the result of the change from light to heavy forms of protein over time. Similarly, we marked newly produced mRNAs in the cells with the nucleoside analogue 4-thiouridine (4sU) (Dolken *et al.*, 2008), so that they could be isolated from the total RNA pool (Fig. 1, right). Comparison of newly synthesised mRNA with pre-existing quantities enabled us to experimentally determine the transcript turnover rate. Close cooperation with the group led by Wei Chen, an expert in sequencing technology, was indispensable in this connection. In all, half-life times for the mRNAs and proteins of more than 5,000 genes were determined in this way.

Proteins are on average more stable and abundant than their corresponding mRNAs

Proteins, with an average half-life of 46 hours, are about five times more stable than their corresponding mRNAs with an average lifespan of just nine hours. Half-life times of proteins ranged from less than an hour to several hundred hours and were thus significantly more dynamic in scope than mRNA half-life times. Using DNA sequencing technology and mass spectrometry we were also able to quantify mRNAs and proteins absolutely. While this process has already been described for mRNAs, we developed a method of our own by which to measure cellular protein concentration. This process, termed intensity-based absolute quantification (iBAQ), makes it possible to rectify the relatively rough intensity values that mass spectrometry provides for proteins and to globally quantify protein concentration across the whole proteome.

Figure 1: Experimental set-up for parallel measurement of protein and mRNA turnover Proteins



To quantify protein turnover we used the SILAC approach, based on the incorporation of isotope-marked, heavy amino acids in proteins. After transferring cells from mice to the SILAC medium, heavy amino acids are incorporated into newly synthesised proteins, whereas pre-existing, light proteins are degraded over time. Using mass spectrometry, the light and heavy forms of thousands of proteins can now be distinguished from one another and cell protein turnover can be calculated from the H/L ratio. To measure mRNA turnover, the nucleoside analogue 4-thiouridine (4sU) was added to the cell culture medium for a certain time. mRNA turnover is calculated by comparing the 4sU-labelled, i.e. newly synthesised RNA, with the unmarked, pre-existing RNA (Chart: B. Schwanhäusser).

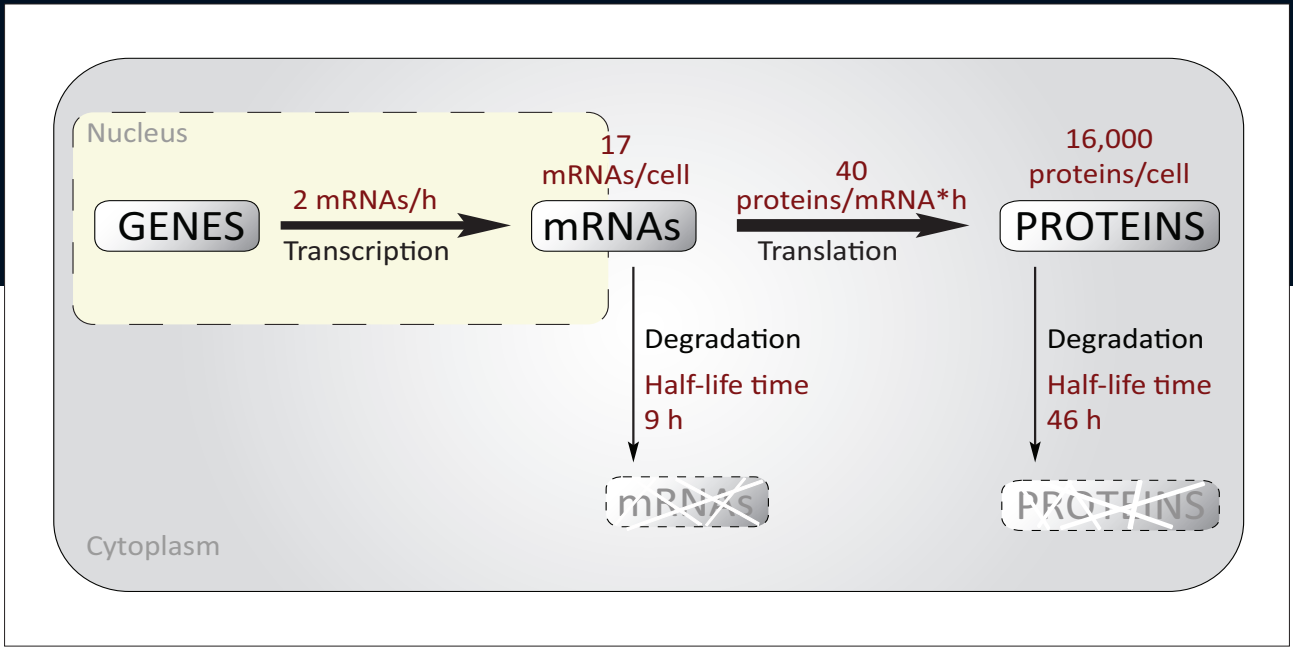


Figure 2: Measuring the gene expression cascade
According to a central dogma of biology, genes are first transcribed into mRNAs in the nucleus. These gene copies then leave the nucleus and are used by ribosomes in the cytoplasm as a blueprint for protein synthesis. We were able, for the first time, to quantitatively record the individual gene expression stages, i.e. transcription, translation, mRNA and protein degradation, and absolute transcript and protein levels of about 5,000 genes in cells from mice. The thickness of the arrows represents the influence of the individual processes on the control of gene expression. The figures reflect median values (Chart: B. Schwanhäusser).

When absolute mRNA and protein quantities were compared, the picture was much the same as for the half-life times. With an average 16,000 copies per cell, proteins are nearly 1,000 times more abundant than their corresponding mRNAs. The number of protein copies per cell ranges from less than 100 to more than 10 million – a much wider range than is measured for transcripts. In other words, this means that, on average, an mRNA serves as the model for the synthesis of about 1,000 proteins. If you look for a direct connection between mRNAs and proteins, you will notice that there is practically no correlation at all where their half-life times are concerned. So mRNA with a short cellular lifespan can code for a stable protein and vice versa. In contrast, absolute amounts of mRNA and protein levels are well correlated. Here we observed a marked correlation that is significantly greater than has previously been described for mammalian cells.

Yet mRNA and protein half-lives are evidently not arbitrarily related because specific combinations of mRNA and protein turnover rates indicate an optimisation of genes towards their biological functions. Dynamically regulated genes such as transcription factors are characterised by short mRNA and protein half-life times, whereas gene products that are more abundant and thereby more "expensive" in energy terms, such as structure proteins, have high mRNA and protein stabilities. These findings are indicative of evolutionary design principles that enable a compromise between energy expenditure and dynamic cell response.

The first global quantification of transcription and translation rates

The turnover rates and absolute quantities we measured were used, with the aid of mathematical modelling, to calculate overall synthesis rates, i.e. transcription and translation rates, for more than 5,000 genes for the first time. The model was developed by Dorothea Busse of Jana Wolf's group, who deals with the mathematical description of biological processes. According to our results, an average gene is transcribed into about two mRNA molecules per hour, with individual genes able to generate up to 100 transcripts in that time. On average, a single mRNA then serves as a blueprint for a protein 40 times an hour. Interestingly, there seems to be a maximum translation rate of around 180 proteins per mRNA and hour. Furthermore, some transcripts have extremely low translation rates, which may indicate that their translation is suppressed in a targeted manner via post-transcriptional regulation mechanisms.

Protein translation is the driving force in controlling the amount of protein in cells

Global measurement of gene expression now enables us to look into which of the four processes (transcription, mRNA degradation, translation or protein degradation) takes the lead in controlling the amount of protein in cells. In the cell, all processes are combined to different degrees in order to adjust protein concentrations precisely to the cell's requirements. Based

on the comparison of protein levels predicted by our model with experimentally obtained protein amounts, we arrived at a surprising finding. Protein translation plays the central role – a much larger one than previously assumed – in controlling the amount of protein. Protein degradation is of minor importance by comparison, at least in the experimental conditions that we studied. This by no means rules out the possibility that the precisely coordinated degradation of proteins plays undoubtedly a fundamental role in certain processes such as the cell cycle. So to simplify matters, it may be said that in our model system the amount of proteins is determined mainly by translation at the ribosome in the cytoplasm.

To summarise, our data can be seen as a first precise and comprehensive census of cell proteins and mRNAs (Fig. 2). This publicly available data can serve as the starting point for many other exciting analyses with a view to clarifying more specific issues. To understand cellular signal processing, for example, it is important to know the quantities of proteins involved. In addition, our data can be mined for frequent sequence motives which serve as degradation signals in mRNAs or proteins. Possibly the most important question is, however, the point at which regulation of the cellular gene expression cascade gets out of control in diseases. The door to dealing with fundamental questions of this kind has now been opened wider.

The research project in brief:

The Global Quantification of Mammalian Gene Expression Control project was undertaken in Prof. Matthias Selbach's group at the Max Delbrück Center for Molecular Medicine (MDC) and at the Berlin Institute for Medical Systems Biology (BIMSB) as part of my PhD thesis. A fundamental part of the project was the close cooperation with the research groups led by Dr. Wei Chen and Dr. Jana Wolf (both MDC/BIMSB). The group led by Prof. Selbach was funded by the Helmholtz Association, the BMBF (NGFN-Plus network Neurodegenerative Disorders – NeuroNet), the German Research Foundation and the European Molecular Biology Organisation (EMBO). I owe a special debt of gratitude to Na Li (sequencing), Dorothea Busse (mathematical modelling), Gunnar Dittmar (protein degradation) and Johannes Schuchhardt (statistics).

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bundled resources – networked expertise

SystemsX.ch – a flagship project of Swiss science policy

by Matthias Scholer

Switzerland aims to be a step ahead in systems biology. This credo has enjoyed widespread scientific and political support in the country for a long time. It was a consensus that made possible the establishment of the structures required for countrywide and cross-institutional collaboration.

Systems biology crosses borders – academic, institutional and financial. For research to be effective in this area of science, disciplines that have previously had little to do with each other must work together. Additionally, systems biology also requires a critical mass of funding and personnel.

These needs were recognised in Switzerland and the course was set accordingly for a nationwide bundling of expertise and resources.

The foundation stone for this ambitious initiative was laid in 2007 with the establishment of the SystemsX.ch research network. What began as a collaboration between three universities now comprises 12 equal partners: Two federal universities, seven cantonal universities and three other research institutions. SystemsX.ch has developed into Switzerland's biggest ever public-sector research initiative and is focused on an area of basic research.

Since 2008 this knowledge network has enabled more than 1,000 scientists to collaborate efficiently on around 100 projects in more than 300 research groups. All of these projects are interdisciplinary in design and require close cooperation between biologists, physicists, chemists, mathematicians, computer scientists, engineers and medical scientists.

"No" to the watering can principle

Between 2008 and 2011, the federal government provided SystemsX.ch with 100 million francs in funding. Money is allocated to the various research projects according to the matching funds principle, which means that an institution only receives research funding if it commits the same amount of funding itself to the project.

Three quarters of this funding went towards 14 large-scale projects which are, of course, SystemsX.ch's flagship projects. These so-called RTD (short for research, technology and development) projects range from work on the processes involved in the development of flies' wings to computer-assisted simulation of metabolic processes (cf. "RTD projects at a glance").

The projects are supported by SystemsX.ch not only financially, but also technologically. SyBIT, the network's own IT and bioinformatics project, provides scientists with standardised data management, processing and archiving, and ensures their freedom of access to this data. In this way all research groups are able to benefit from existing results and measurements.

Nurturing the next generation, risk and the private sector

Interdisciplinary dissertations, interdisciplinary pilot projects and bridge-to-industry projects complete the SystemsX.ch portfolio.

While funding of interdisciplinary dissertations provides targeted assistance to help promote young systems biologists, interdisciplinary pilot projects go for risks. This is an area in which scientists can embark on projects that would not normally receive research funding because the prospect of scientific success seems too slight. The recognition gained is all the greater if a breakthrough is nonetheless achieved.

Key figures in SystemsX.ch:
Dr. Daniel Vonder Mühll (Managing Director) and Professor Ruedi Aebersold (Chairman of the Scientific Executive Board)
(Photo: Rahel Schumacher, IMSB ETH Zurich).

To promote cooperation with the private sector, SystemsX.ch has in recent years launched 14 bridge-to-industry projects in which university research groups collaborate closely with pharmaceutical and biotechnology companies or spin-offs. Setting up public-private partnerships of this kind is demanding in view of the different cultures and objectives, but it is indispensable for long-term research success.

Constant quality control and competent decision-makers

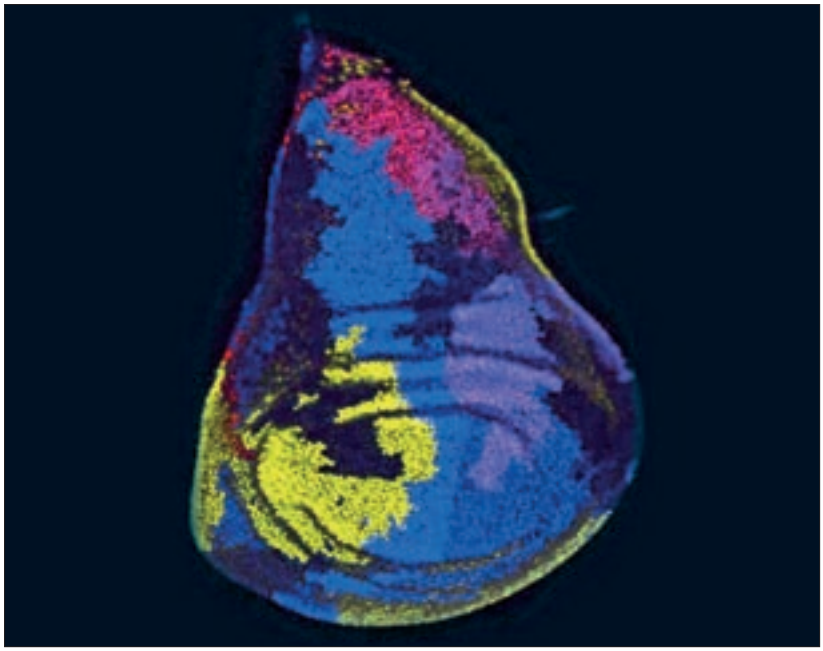
The Swiss National Science Foundation (SNSF) monitors the quality of research work. It assesses not only the major research projects and interdisciplinary dissertations but also regularly evaluates the initiative's overall progress. This independent scrutiny ensures and promotes international competitiveness.

The choice of interdisciplinary pilot projects, however, remains the prerogative of the Scientific Executive Board. It is the operational management body of SystemsX.ch and its members are scientists from the network's partner institutions. Strategic management is the responsibility of the Board of Directors, consisting of the presidents, rectors and directors of all of the participating institutions.

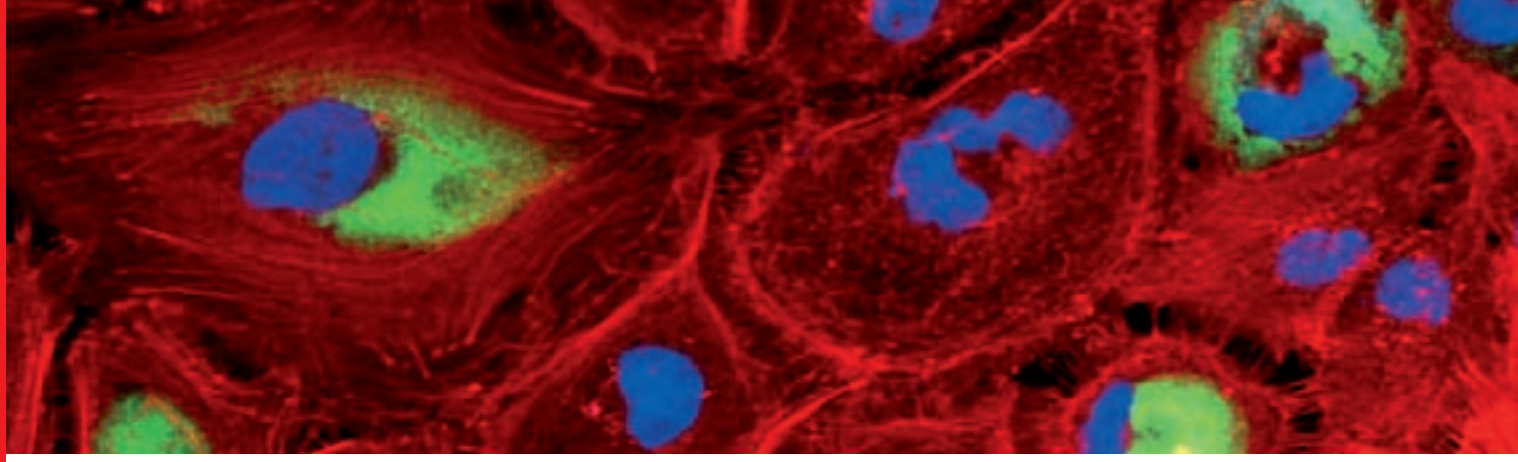
Full speed ahead

SystemsX.ch is on course for success, with the necessary network structures in place and highly promising research projects already underway. For the forthcoming phase of consolidation from 2012 to 2016, the Swiss National Science Foundation (SNSF) has recommended that the Swiss Federal Council and Parliament allocate a further 120 million francs to SystemsX.ch. In the years ahead the initiative will consolidate the processes on which it has embarked and intensify its bundling of expertise – always with a view to being among the world's best in systems biology.

WingX:



A wing disc from which the wing develops in the course of metamorphosis. Individual cells were genetically labelled at an early stage of development. The resulting cell clones show the pattern of cell division in the course of development (Photo: WingX).



InfectX:
Intracellular *Brucella* – bacteria that cause Malta fever or brucellosis (green) – in human cells (cytoskeleton: red; nucleus: blue) (Photo: InfectX).

The SystemsX.ch RTD projects at a glance:

BattleX – uses the *Shigella sp.* bacterium that triggers dysentery in 160 million people all over the world to examine which metabolic interactions take place between human host cells and the bacteria and which of these interactions might serve as target points for new antibiotics.

Cell Plasticity – investigates the system of regulatory networks that makes cellular differentiation possible in mammals. Its main focus is on understanding and modelling the mechanisms that take place in the sequence-specific binding of transcription factors and on the dynamics of the epigenetic code in the entire genetic material.

CINA – develops methods by which individual cells and their interiors can be mapped in the nanometre range. One approach makes it possible to characterise the entirety of a cell's proteins, or proteome, by means of visual high-throughput methods. It is used, for example, for systems biology analysis of the protein composition and its structural conformations in the context of cellular changes that are of relevance for Alzheimer's or Parkinson's disease.

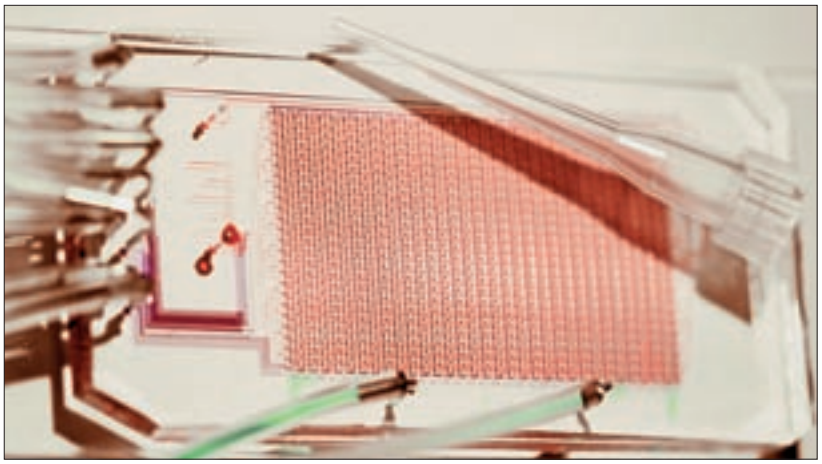
CycliX – Cyclic regulatory circuits are fundamental building blocks in every organism. CycliX seeks to understand three of these circuits and how they interact: The circadian rhythm, cell division and nutrient-response cycles.

DynamiX – investigates the dynamics of proteins and their quantitative measurement. Scientists are particularly interested in how much protein is produced in a cell at which point in time and when and how these proteins interact.

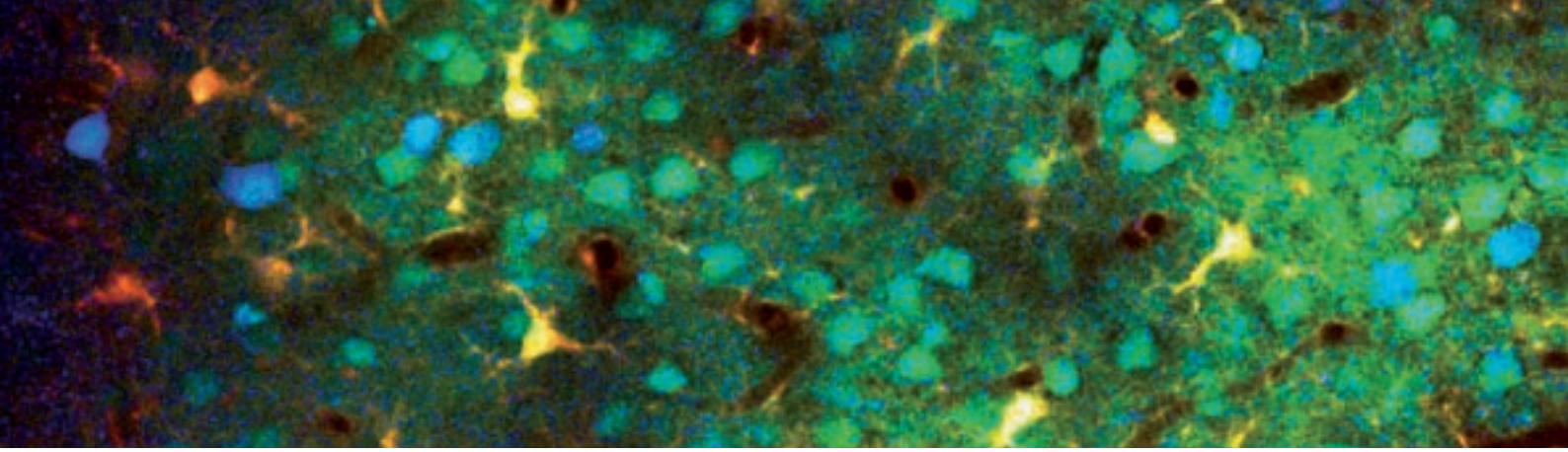
InfectX – This project aims to identify all the components that are relevant for the entry of a virus into host cells and then to design mathematical and computer-assisted models to identify novel approaches for anti-infectives.

LipidX – Lipids can fairly be said to be the least understood cellular biomolecules and an unappreciated part of a cell's entire metabolic properties. The aim of this project is to gain an understanding of the function, the design and the distribution of these complex structural components in cells.

DynamiX:



The microfluidic device developed as part of the DynamiX project to characterise in vitro protein interaction (Photo: S. Maerkl).



Neurochoice:
A cross-section of the brain of a transgenic GAD67-GFP mouse using two-photon laser microscopy in which GABAergic cells express GFP (blue). Astrocytes were selectively dyed orange-yellow with sulforhodamine 101. In addition, all cells were dyed using the calcium-sensitive Oregon Green BAPTA-1 dye (Photo: Neurochoice).

LiverX – aims to find out why a healthy liver cell responds to insulin while an insulin-resistant cell does not. The research scientists hope their findings will provide fresh stimulus for treating diabetes.

MetaNetX – focuses on models for metabolic networks, especially their automatic generation and use to annotate genomes and for simulation, such as to gain a better understanding of plant metabolism.

Neurochoice – In everyday life we are constantly making decisions. Some are considered, others are more instinctive, knee-jerk reactions. The research scientists in this project are trying to find out what processes go on in the brain during decision-making, both at the level of neuronal circuits and in the networks of different parts of the brain.

PhosphoNetX – has set itself the target of understanding the phosphorylation of proteins and the regulation that it controls. Conclusions can be made from the findings about the dynamic processes that go on in a cell.

Plant Growth – deals with the question of how a system of chemical and mechanical processes regulates plant growth. Innovative experiments and their computer simulation play an important part in this process.

WingX – investigates the processes that occur in the development of the wing of the fruit fly *Drosophila melanogaster*. The findings should help scientists to gain a better understanding of how human organs develop and enable them to make computer simulations of the process.

YeastX – examines the regulatory processes that take place in yeast cells in order to develop a fundamental modelling concept to clarify molecular biology phenomena.

For further information, please visit:
www.systemsx.ch

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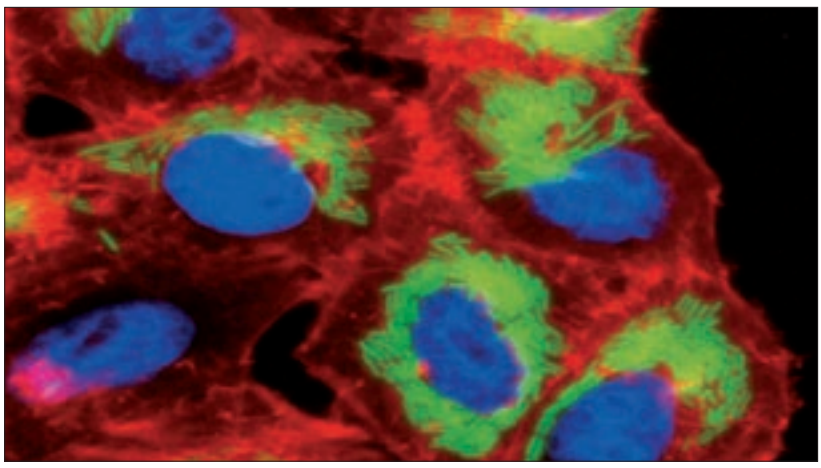
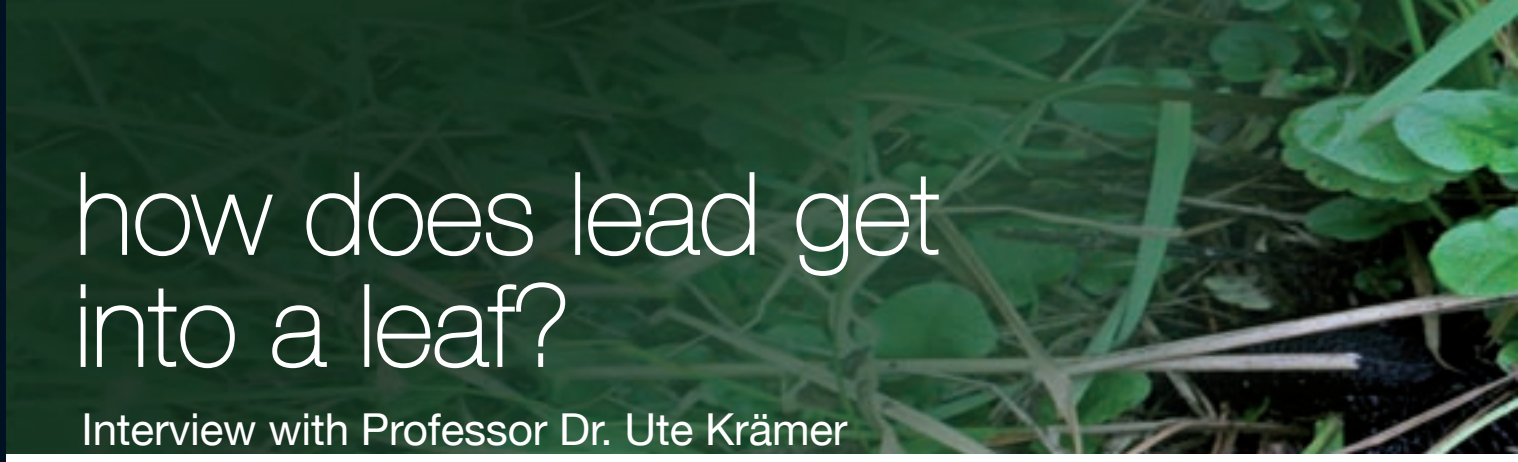
BattleX:



Human cells (nucleus: blue; actin: red) infected with *Shigella* (green) (Photo: BattleX).



how does lead get into a leaf?

Interview with Professor Dr. Ute Krämer

Department of Plant Physiology, Ruhr University Bochum

Biochemist Ute Krämer of the Ruhr University Bochum has dedicated her research to plants in order to gain insights into the evolution and adaptation strategies of these survivalists. For this, she is studying the metabolism of metal hyperaccumulators, fascinating plants that during evolution have acquired the species-wide ability to accumulate extraordinary high concentrations of heavy metals in leaves without suffering any damage. Discovering more about these amazing vegetal devourers of metals also holds the promise of interesting new areas of application, for example the use of plants to decontaminate soils polluted with heavy metals.

Professor Krämer, can plants have something like a ravenous appetite?
I wouldn't really say that. I think the concept of a ravenous appetite is more appropriate for humans.

Yet the plants you are investigating do have a very special and unusually large appetite for heavy metals. They do not shy away even from the highly toxic cadmium.
Yes, this is true. That is why these plants are called metal hyperaccumulators.

What exactly does that mean?
Metal hyperaccumulators are plants that accumulate in their leaves enormously high concentrations of heavy metals such as nickel, zinc or cadmium, and possibly even lead. Such quantities of internally accumulated heavy metals would be fatal for all other organisms. The model species that we focus on in our work, *Arabidopsis halleri*, can accumulate more than 2% of leaf dry biomass in zinc and more than 0.05% in the highly toxic cadmium.

Why do plants do this kind of thing?
It's certain that they acquired this uncommon characteristic during the course of evolution. The high doses of metal might protect them from predators or plant diseases. And maybe metal-rich soils, in which hardly any other species can survive, are just a suitable ecological niche. The selective advantage resulting from plant metal hyperaccumulation has yet to be explained conclusively.

Where do these unusual plants occur? And how many are there?
More than 500 metal-hyperaccumulating plant species have been discovered so far. They are not easy to find. People have looked for them all over the world, as yet mainly in locations contaminated with heavy metals or naturally containing large quantities of heavy metals of geological origin. We now know that metal-accumulating plants also occur in locations without considerable heavy-metal contamination. However, the search for them has not been as intense at such sites, so that we have certainly not yet identified all metal hyperaccumulator plants.

When and where did the first metal-eating plant show up?
In the nineteenth century, in Germany, at a site contaminated with heavy metals. Using chemical and analytical methods that were quite basic in those days, scientists managed to ascertain that the leaves of these plants contained very high metal concentrations.

How did you, as a biochemist, encounter hyperaccumulators?
It was by chance. I wanted to do some work on plants for my doctorate, and on an ecologically relevant topic. I went to Oxford on a Rhodes Scholarship, and the professors in the Department of Plant Sciences spent a whole day speaking with me. It turned out that they didn't have much to offer in this topic area. However, immediately afterwards I received a message from one of the professors I had spoken to, Andrew Smith, saying that he had a friend named Alan Baker ... who had these heavy-metal hyperaccumulating plants ... and that perhaps I could do something with them. That's how it happened. And I was immediately filled with enthusiasm.

What is it about a few hundred rather strange plant species that fills you with such enthusiasm?
At that time, I was fascinated by this amazing capacity of these unusual plants, and I wanted to understand how it functions at the biochemical level. However, working with a biological exception is not my main interest. True, the phenomenon of metal

Arabidopsis halleri **competing with grasses and other plants in a non-contaminated area in Malmedy, Hautes Fagnes, Belgium** (Photo: Ricardo Stein & Ute Krämer).

hyperaccumulation has great audience appeal, because many people have never heard of it, but for me these plants are first and foremost models for answering questions about evolution of a physiological trait, distinct from the morphological traits that have classically been studied in this context. Good models that permit research of this kind are rare, but the physiological traits of metal hyperaccumulation and associated metal hypertolerance are very well suited for this purpose.

What have you been able to learn about vegetal metal-eaters so far?
First, we asked which genes are involved in the metal hyperaccumulation phenomenon. Applying modern genomics, we were able to identify a number of candidate genes. Now, we are increasingly in a position to demonstrate which functions individual genes fulfil. This is providing us with progressively precise information how the complex network of metal homeostasis is characteristically modified in these hyperaccumulators to account for the dramatic phenotypic outcome that we are interested in.

Do you have an example of the function of one of these genes?
One example is the so-called *HMA4* gene, which encodes a transporter of heavy metals, a protein that can transport zinc and cadmium. The protein is present in the plasma membrane of certain cells and dispatches metals out of these cells into the xylem vessels for root-to-shoot transport driven by transpiration. In plants which hyperaccumulate metals, we find much larger quantities of the protein. Thus the plants accumulate metals specifically in their above-ground tissues. From an evolutionary point of view, our work suggests that a combination of *cis*-regulatory mutation and gene copy number expansion contribute to high levels of *HMA4* expression in *A. halleri*.

How much is known in general about such molecular material-distribution pathways in plants?
We know many individual proteins and their functions, but we do not have the big picture yet. How do these proteins interact at the level of the entire plant, and what are the binding forms of metals at each stage along their transport pathway in the plant? And how does the metal homeostasis network interact with other processes, such as growth and development? There are still many gaps in our knowledge. Above all, in order to achieve an informative synopsis, knowledge of functions specific to different cell types is very important, that is, understanding the division of labour within the plant. In recent years, there have been enormous methodological advances in plant research. The linking of different types of large experimental datasets and computerised mathematical modelling are developing very rapidly. To deal with the increasing complexity, computer-based approaches are playing an important role in enabling systems biology approaches to generate hypotheses in the form of working models that can then be verified experimentally.

What goals would you like to achieve with your work in the near future?
First, we would like to reach a comprehensive understanding of how the metal balance interacts with other processes, such as the development of the plant, its growth, and photosynthesis, for instance. After all, metals are not exclusively toxic. A number of metals perform vital tasks in every cell of all organisms. Iron, copper and zinc, for example, have very potent catalytic properties that have been recruited by numerous critical biochemical reactions during evolution. Thus, in plants, these essential metals must be transported to just the right place in the plant body where they are needed, and in precisely the right amounts. We do not yet understand fully how that functions in any organism. Ultimately, we want to understand the complete processes that enable plants to construct something like a photosynthetic apparatus, for example, which requires particularly large quantities of metals. Our second, rather overarching, goal is to reach a comprehensive understanding of evolutionary adaptation in plants, at all levels of investigation: Which genes are involved? Which proteins are encoded by these genes? What do these proteins do, and where? What are the results at the metabolic level and in the "visible" plant? Which types of mutations play an important role in evolutionary adaptation of plants in general? And how does plant evolution take place in "real time"?

Arabidopsis halleri **in a heavily contaminated region in Miasteczko Śląskie, Poland, approximately 200 metres from a zinc smeltering plant. Only a few specialized plants that are highly tolerant to heavy metals are able to survive in this severely polluted area.** (Photo: Ricardo Stein & Ute Krämer).

How does the biological peculiarity of metal hyperaccumulation fit in here?

In order to obtain fundamentally novel insights about plants, we are investigating them from a particular angle. Our angle is metals, because they are both extremely important and also highly dangerous for life. This perspective is uncommon, and consequently we can expect completely novel insights. Now, after more than a decade of research, this initial expectation is being fulfilled in more interesting ways than I ever anticipated.

Of what use can this type of basic research be?

Until now, little attention has been paid to the fact that an unfavourable distribution of metals within a plant can severely limit the productivity of a plant variety. Therefore, we can contribute findings that are important knowledge for plant breeding. I believe that in general, basic knowledge about plant evolution and adaptation, which can be generated in a much more comprehensive manner today than ever before, holds great potential for plant breeding. More specific applications arising directly from the subject of metal accumulation are already foreseeable. For example, many people all over the world suffer from nutritional iron or zinc deficiency. This could be compensated by a higher metal content in food. It would, for example, be worthwhile using modern breeding methodology to increase the zinc and iron content of crops. For example, grain micronutrient contents have been observed to continually decline in cultivated wheat varieties since the 1950s. We do not know the exact reason why. A second area of application arises from the fact that the contamination of soils with heavy metals, especially cadmium, has been rising steadily throughout the World since the onset of industrialization. Via contaminated plants, heavy metals enter the food chain and finally accumulate to harmful levels in humans. The intake of cadmium via almost perfectly normal food is already having a demonstrably negative effect on the health of the European population. People who consume food containing even slightly enhanced quantities of cadmium over an extended period encounter a strongly enhanced risk of kidney failure and osteoporosis during the course of their lives. We would like to know how to breed plants that absorb a sufficient quantity of metals such as zinc and iron, but exclude cadmium although it has similar chemical properties. However, we still know too little about which genes control the discrimination between different metals along their pathways of movement through the plant. Increasing arsenic contamination of rice is another major global problem. Here it is desirable to breed cultivars that accumulate less arsenic in their grains. This requires precise knowledge of the pathways of movement of arsenic into rice grains, of the genes governing these pathways and gene variants altering them.

Another application is using metal-eaters to clean up soils contaminated with heavy metals. Sow, harvest, problem solved?

Almost 20 years of research have gone into the cleaning of soil utilizing plants' natural abilities to accumulate inorganic con-

Arabidopsis halleri population:

Large *Arabidopsis halleri* population in a non-contaminated region 1,550 m above sea level in Viano-Zavena, Switzerland (Photo: Ricardo Stein & Ute Krämer).

taminant ions. Technologies for plant-based soil clean-up, called phytoremediation, are already on the market. They include, for example, a fern that hyperaccumulates arsenic and is currently sold in the United States. It is certainly wrong to advocate hyperaccumulating plants as a panacea for all the problems caused by heavy metals, although they could certainly prove useful for specific applications. Moderate cadmium contamination can, for example, arise from multiple application of sewage sludge as a fertiliser. By sowing and harvesting cadmium-accumulating plants once or twice one might be able to reduce the cadmium contamination in the topsoil to a level below a critical value. However, phytoremediation cannot be used to clean up massive levels of pollutants within realistic timescales, as can be found, for example, in former mining areas and in the floodplains of the rivers downstream.

You are still very young but have already worked at many research institutions in Germany and abroad. What do you need to feel comfortable as a researcher?

I feel comfortable in an interactive, international and interdisciplinary environment with flat hierarchies. Of course, there also has to be an appropriate and flexible infrastructure and financial resources that allow me to pursue ambitious long-term research goals using contemporary methods. Unfortunately, in German universities administration, bureaucracy and teaching duties take up a lot of your working hours, so that research is largely done in your spare time. This is not optimal for scientific productivity. The situation has certainly improved a lot in recent decades, but in the past few years there has been a noticeable trend for the worse. The newly introduced German Bachelor's and Master's courses, which require a lot more supervision and administration, play a big part in this. Universities simply have insufficient long-term teaching staff, and ultimately, this ends up operating at the expense of research. Biological sciences are subject to rapidly intensifying methodological and conceptual dynamics. There is enormous growth in the number of researchers worldwide, research productivity and in the magnitude of insights gained, and precisely this makes it so interesting now. This also means that as a scientist today, you have to invest more time and effort in order to perform at cutting-edge level.

What is your biggest challenge right now?

My biggest challenge at present is to meet all the needs of my two small children and family while continuing to work towards my professional goals.

Interview by Claudia Eberhard-Metzger.

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Site of a former mine in Lautenthal, Harz Mountains, Germany:

Large *Arabidopsis halleri* population and other metal-tolerant plants in a severely polluted region contaminated with heavy metals (Photo: Ricardo Stein & Ute Krämer).

e:Bio – innovations competition systems biology

New 'roof concept' for systems biology research funding from the Federal Ministry of Education and Research (BMBF)

by Bernhard Gilleßen

"The answers to our problems arise from the future and not from the past," the German biochemist and environmental scientist Frederic Vester (1925–2003) once said. With its various applications, the systems biology research approach provides ample scope for future visions. In this key technology, application and demand are at the same time perspective and challenge. It is all the more remarkable that, within just ten years and through good science as well as a forward-looking research funding strategy, impressive progress has already been achieved.

Ever since the Federal Ministry of Education and Research (BMBF) announced its call on "Systems of Life – Systems Biology" in 2001, it has actively shaped the framework conditions for this new research approach with a series of thematically, structurally and temporally coordinated initiatives (cf. systeme des lebens (2010), systembiologie.de 1:8–11). Only recently, the BMBF´s Advisory Board on Systems Biology confirmed that German scientists do not only have an internationally competitive edge but also represent very desirable scientific project partners.

A major objective of the latest BMBF funding activity, the e:Bio - Innovation Competition Systems Biology, is to consolidate this good position. In the medium term, through ongoing support of excellent science, research infrastructure and a cutback of research barriers, an optimal starting point for the further and sustainable development for this research approach will be provided. This is why the BMBF call, announced at the beginning of 2011 (http://bmbf.de/foerderungen/15679.php), focuses on training young investigators (Module III), bridging basic research and application (Module II) as well as incorporating new stimuli, ideas and innovations into systems biology (Module I). Due to this broad scope and the temporal continuity, with three subsequent calls between 2011 and 2013, e:Bio is the BMBF´s 'roof concept', with which systems biology research can be easily identified with.

In 2011, the record number of 170 individual and joint project proposals were submitted in response to the call. As expected, the "ideas competition national" was the main focus of interest with more than half of the applications (91 in total). However, the "young investigators" and "transfer" modules were also popular, accounting for 38 and 41 project proposals respectively. The high level of participation by small and medium-sized enterprises (SMEs) and large-scale industry, with more than 70 coordinators and project partnerships, is an equally good indication of the high transfer potential and challenging expectations of systems biology. The enormous scope of submitted project ideas, extending well beyond the previously funded range of topics, is further evidence of the universal applicability of this research approach.

To meet the requirements of this large number and scientific diversity of applications, the scientific assessment was undertaken by an international reviewing board consisting of no fewer than 35 selected experts. This represents another record for a German systems biology funding initiative. In a two-stage

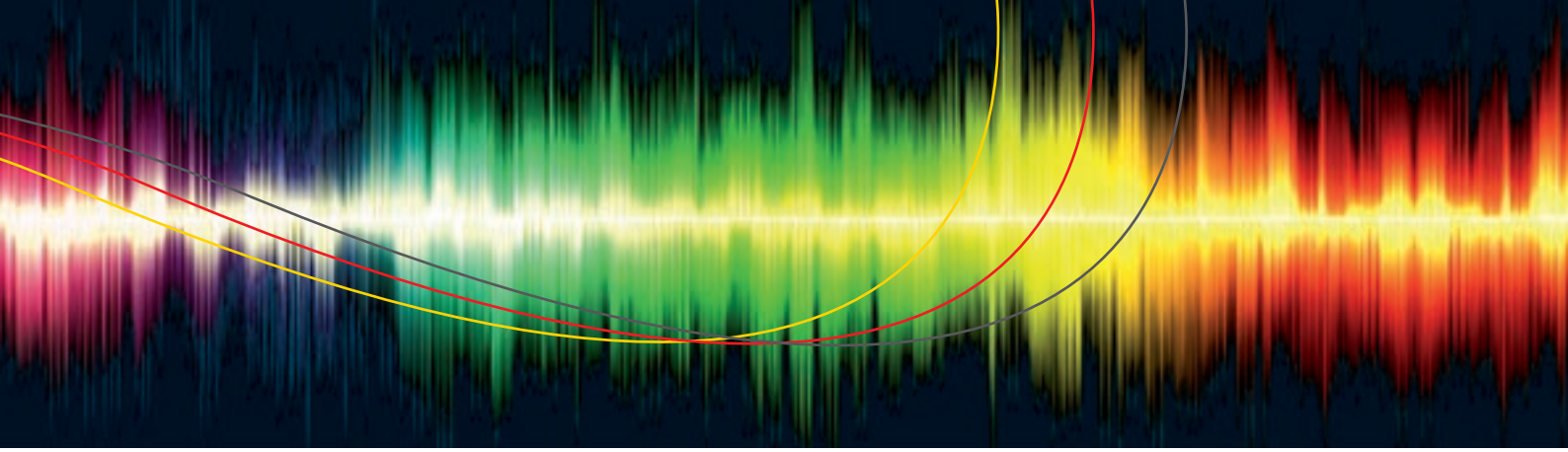

Image: © sander24 – Fotolia.com

reviewing process starting with a preliminary vote in writing, followed by an in-depth oral discussion session, the panel prepared an evaluation report for each project. This initial vote by the panel of experts represented the basis for a final ranking concluded by the Systems Biology Advisory Board, who also took strategic aspects for research development into account, before submission to the Ministry.

By inviting 35 individual and joint projects to formally submit an application, research consortia with an estimated funding volume of EUR 65 million were selected in the first round - a remarkable amount for a systems biology initiative. Starting with the actual scientific work in 2012/2013, all projects will be part of the multi-layered e:Bio 'roof concept'. The www.ebio-initiative.de website will not only make the initiative accessible to the general public, it will also provide an opportunity to present specific projects, their work and their latest findings. Regular status seminars will ensure the scientific quality of the projects and help make scientific exchange and networking possible.

With the e:Bio - Innovation Competition Systems Biology, the BMBF has demonstrated a significant and long-term commitment to this specific research approach. The first round of funding will initiate a groundbreaking process, in which new standards will be created for tangible future systems biology.

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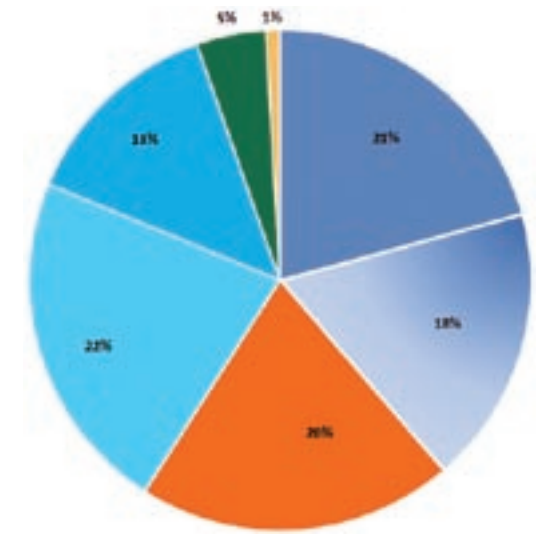
News from the BMBF

The Federal Government is firmly committed to education and research

The promotion of education and research is a vital investment in the economic future of a country like Germany, which has few natural resources. The Federal Government's budget once again provides for a spending increase in these areas in 2012. The BMBF's total budget is to be increased by more than 11 percent to 12.9 billion euros. In the words of Federal Minister of Education and Research Annette Schavan: "The Federal Government is focusing on education and research as key policy areas. Thanks to this strategy, Germany has emerged stronger from the world-wide economic and financial crisis. Investing in people's minds is the only way to nurture and develop existing potential."

BMBF (departmental budget 30) – areas of responsibility 2012
Departmental budget 30 – €12.941 billion



- Knowledge-oriented basic research across different programmes
- Public services research and development
- Technology and innovation funding
- Building of institutions of higher education and special programmes directed mainly at universities
- Federal Training Assistance Act (BAföG)
- Other, non-R&D-relevant education expenses (excluding BAföG)
- Ministry, including pensions

Project funding will remain a key element of this policy. Adopting a clear global approach, the BMBF is concentrating on climate and energy, health and nutrition, mobility, security and communication. For example, approximately 700 million euros have been earmarked up to 2015 for the development of German Centres for Health Research to improve the prevention and treatment of common diseases.

Further information is available at:
http://www.bmbf.de/en/96.php

The Public Dialogue on High-Tech Medicine

The Federal Ministry of Education and Research is providing a forum for members of the public to raise their questions and wishes concerning the future of medicine in the political sphere. A discussion forum with a focus on telemedicine, neuronal implants and palliative and intensive medicine has been set up on the Internet at www.buergerdialog-bmbf.de. In addition to the on-line dialogue, one-day public conferences are being held where approximately 100 participants discuss the future use of medical technologies with experts from research, business and politics. The findings will be submitted to Federal Minister Annette Schavan in the form of a civic report with policy recommendations. The public dialogue on "High-Tech Medicine" is part of a comprehensive exchange of views between members of the public, the scientific and business communities and politicians which the BMBF is organising to deal with various emerging technologies over the next four years.

Further information is available at:
www.buergerdialog-bmbf.de/

BÜRGER DIALOG

Federal Cabinet adopts 6th Energy Research Programme

Ensuring a reliable, affordable and environmentally sound energy supply is an important objective of Germany's new energy strategy. The development and application of innovative energy technologies play an important role in this context. The

Photo: © andrea lehmkuhl – Fotolia.com

Federal Government's 6th Energy Research Programme contains guidelines and priorities for its funding policy in five important areas: Infrastructure, energy efficiency, renewables, safety, waste disposal and radiation research as well as systems and acceptance research. The newly established Energy and Climate Funds will account for a large proportion of a total budget of around 3.4 billion euros between 2011 and 2014. Federal Minister of Education and Research Schavan and her cabinet colleagues Dr. Philipp Rösler (Economics and Technology), Dr. Norbert Röttgen (Environment, Nature Conservation and Nuclear Safety) and Ilse Aigner (Food, Agriculture and Consumer Protection) are pooling the core expertise of different government departments in order to address important energy policy issues within the framework of joint funding initiatives.

Further information is available at:
www.bmbf.de/press/3136.php

Battery production is leading Germany towards electromobility

The automobile industry is facing fundamental challenges on the road to electromobility as a technology for everyday use. Germany has cleared another important hurdle towards becoming a leading global provider. The BMBF is paving the way for an efficient and affordable storage technology by funding a pilot production facility for lithium-ion batteries in Ulm. Federal Minister Annette Schavan has agreed with the Lithium-Ion Battery Competence Network (KLiB) to set up such a production facility, thus fulfilling a central demand of the National Platform E-Mobility. The BMBF's commitment to the KLiB complements the Lithium-Ion Battery Innovation Alliance 2015, which it initiated in 2007.

Twenty-five companies and organisations are involved in the KLiB and are contributing their long years of internationally outstanding expertise. Before the end of the year, planning will get underway for a facility to research and optimise the manufacture of lithium-ion cells. Current major challenges are the transfer of newly developed production processes, materials, components and plant parts to the industrial manufacture of batteries for electric vehicles that are close to series production.

Further information is available at:
www.bmbf.de/press/3029.php

Photo: © sebastianreuter – Fotolia.com

Unique research structures help patients

Cancer, cardiovascular diseases, metabolic diseases, infectious diseases, lung diseases or neurodegenerative diseases are now termed common diseases because the number of people affected has increased steadily in recent years. The BMBF is addressing this challenge by establishing the German Centres for Health Research (DZG). By 2015, the Ministry will have made 700 million euros available to optimise research conditions, accelerate the transition from research to practical use and improve prevention and therapy.

Expertise from more than 120 university and non-university research facilities will be united at 39 locations. The German Centre for Neurodegenerative Diseases (DZNE) and the German Centre for Diabetes Research (DZD) already began their work in 2009 and are now being joined by the German Centre for Cardiovascular Research (DZHK), the German Centre for Infection Research (DZIF), the German Centre for Lung Research (DZL) and the German Consortium for Translational Cancer Research (DKTK).

The German Centres for Health Research are a core element in the Federal Government's Health Research Framework Programme, which was adopted in 2010. "The Centres are the only ones of their kind in the world. They have the potential to put Germany into the lead in health research in Europe and maybe even in the world," said Federal Minister Schavan at the DZG presentation in Berlin.

Further information is available at:
www.bmbf.de/press/3109.php

Federal Government boosts research at medical technology SMEs

Since 2007, the BMBF's KMU-Innovativ programme has been making it easier for small and medium-sized enterprises (SMEs) to access research funding, thereby supporting cutting-edge research in Germany.

The seven areas of technology that are already receiving support – civil security, biotechnology, information and communications technology, nanotechnology, optical technologies, production technology, and resource and energy efficiency – are now being joined by the area of medical technology under the "KMU-Innovativ Medical Technology" programme.

Photo: © Gilles Paire – Fotolia.com

Speaking at the Future of Medical Technology Conference in Berlin, Helge Braun, Parliamentary State Secretary at the BMBF, announced that 10 million euros a year are being made available for this new funding programme. “We want to speed up innovation processes, strengthen the medical technology industry and improve patient care,” he said. Small and medium-sized enterprises (SMEs) are strong pioneers of technological progress in many areas.

Further information is available at:
www.bmbf.de/press/3115.php

Strengthening agriculture in the fight against famine

The increasing frequency of extreme weather conditions and the growing scarcity of fertile land stand in sharp contrast to the need to feed the world’s rising population. Since the turn of the millennium, food prices have risen, often leading to famine and political upheaval, especially in developing countries. “Securing a sustainable supply of food for the world’s population is a central task for the future and a global obligation that can only be met through joint research endeavours. This is an area in which Germany must assume international responsibility,” says Federal Minister of Education and Research Schavan.

The BMBF’s "GlobE – Global Food Security" funding initiative actively supports the worldwide development of sustainable and efficient agriculture to ensure food supplies. Agricultural research topics are to be identified along the entire value chain and studied within the framework of interdisciplinary and international cooperation based on regional needs assessments in Africa. The aim is for German and African partners to build new bridges between highly developed cultivation technologies and traditional techniques, also taking existing local knowledge into consideration. The deadline for funding applications was October 2011.

Further information is available at:
www.bmbf.de/press/3124.php

Contact

Further information about these and other interesting areas of the High-Tech Strategy for Germany is available at:
www.hightech-strategie.de

BRAUNSCHWEIG'S SYSTEMS BIOLOGY CENTRE BRICS GETS GOING

The Technische Universität Braunschweig and the Helmholtz Centre for Infection Research found a novel joint research centre

BRaunschweig Integrated Centre for Systems Biology, BRICS for short
is the label under which Braunschweig scientists from very different disciplines have joined forces to bring forward systems biology. By founding BRICS, the Helmholtz Centre for Infection Research (HZI) and the Technische Universität Braunschweig (TU) are bundling local expertise in systems biology and bioinformatics. Three HZI departments and five TU institutes from three different faculties are taking part in the research centre. Its aim is to develop genuine interdisciplinarity.

Systems biology as the basis of modern health research
At BRICS, systems biology is used to understand complicated infection processes and to improve their treatment. This includes the development of new active agents and their biotechnological production processes along with the optimisation of existing therapies and applications. In terms of methods, bottom-up high-throughput technologies are combined with reductionist top-down approaches.

BRICS is part of the Translation Alliance in Lower Saxony.

With its contribution to the development of new therapies, BRICS represents an important element in the Translation Alliance in Lower Saxony (TRAIN), which links biomedical centres in the Braunschweig-Hanover research region and has the aim of developing active agents in tandem (Source: Hurtig Design).

Diagram: UFZ

These are complemented by single-molecule microscopy and time-lapse films and their subsequent image analysis. Experimental determination of the relevant biological data of a cell and its bioinformatic evaluation finally makes integration into mathematical models possible. Repeated experimental testing of model predictions and iterative adaptations of the theoretical models lead to a deeper understanding of the underlying biological processes. That opens up, as shown in the example on the facing page, future-oriented perspectives in the field of medical translation – from new active agents with tailor-made production processes to new or optimised approaches to therapy.

With support from the state of Lower Saxony, the Technische Universität Braunschweig has commissioned a new building for BRICS:
At a cost of EUR 26 million, a systems biology centre containing experimental labs, offices and internship facilities is under construction on the TU campus.

The groundbreaking ceremony is scheduled for 2013, so the Braunschweig systems biologists will have to be patient for a while. But they are already looking forward to planning their own labs and offices. As Christoph Wittmann put it, welcoming everyone to the first construction meeting, "I am delighted to be here!"

Model of the new BRICS building

Image: © STEFAN LUDES ARCHITEKTEN

The BRICS team (from the left): Prof. Dr. Dietmar Schomburg, Bioinformatics and Biochemistry; Prof. Dr. Philip Tinnefeld, NanoBioSciences; Prof. Dr. Michael Meyer-Hermann, Systems Immunology; Dr. Ida Retter, Coordinator; Prof. Dr. Lothar Jänsch, Cellular Proteomics; Prof. Dr. Katharina Riedel, Microbial Proteomics; Dr. Robert Geffers, Genome Analytics (here represented by Dr. Michael Jarek); Prof. Dr. Christoph Wittmann, Biochemical Engineering; Prof. Dr. Dieter Jahn, Microbiology (Source: Press and Communications, TU Braunschweig).

Photo: UFZ

Can therapies be optimised on the computer?

Model integration makes it possible to predict effects

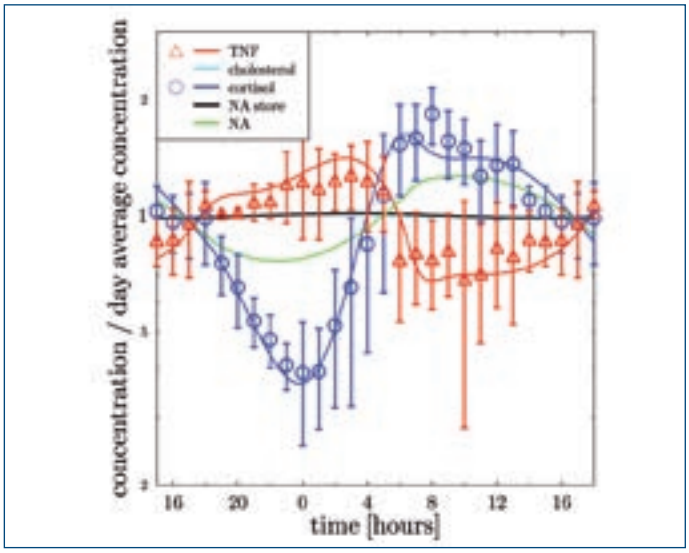
One aim of the BRICS scientists is to improve the treatment of diseases with the aid of mathematical models. Effective therapies have been identified for many diseases, such as cancer or chronic inflammatory diseases, but defining an optimal therapy protocol is not easy. How, for instance, is radiation treatment of tumours to be undertaken? Is it better to have fewer sessions and a higher dose or more sessions and a lower dose? It quickly becomes apparent that empirical medicine will take many years to draw up an optimal protocol for every therapy. Mathematical modelling of complex systems can intervene at this stage and be instrumental in the optimisation of therapies, as the following example shows. Rheumatoid arthritis is an autoimmune disease that develops on the borderline between the body's nervous, endocrine and immune systems. These systems are not independent of each other. A focus of inflammation first activates the immune system and sends signals to the central nervous system, which regulates release of the hormone cortisol. That in turn has an anti-inflammatory effect. Rheumatoid arthritis is a chronic inflammation of the joints. One of the classic therapies is to administer cortisol with the aim of reducing the inflammation and giving the patient some relief.

Patients with rheumatoid arthritis complain of joint pain in particular in the morning. Prof. Michael Meyer-Hermann of the Helmholtz Centre for Infection Research (HZI) and the Regensburg rheumatologist and endocrino-immunologist Prof. Rainer H. Straub made use of this observation by investigating the circadian changes in markers of the three subsystems and replicating them quantitatively in mathematical models (see Fig. 1). This enabled them to make computer simulations of cortisol therapy. The amazing result was that the same quantity of cortisol is three times less effective as an anti-inflammatory in the morning than at midnight.

This prediction has since been confirmed in laboratory experiments and is currently finding its way into medical practice. In clinical practice, patients have hitherto been given cortisol after they got up, which is when it is least effective. The effect of a recognised therapy has thus been increased threefold by the results of mathematical models.

The researchers at the HZI believe that optimising therapies by means of mathematical models has enormous potential. In particular, this approach opens up the possibility of optimising therapies not just universally, as in this case, but for each individual patient. This step in the direction of individualised therapy is the decisive challenge that faces modern medicine.

Figure 1: Changes in nervous, endocrine and immune system markers during the course of the day.



The hormone cortisol (blue) reaches its lowest daily level at midnight (0:00 hours), whereas the immune messenger TNF (red) is at its highest level in the hours of the early morning (04:00 hours) and at its lowest when people get up (at 08:00 hours). The simulated neuroendocrine immune network (lines) is compared with measured data (symbols). TNF – tumour necrosis factor; NA – noradrenaline. Reference: Meyer-Hermann, 2009: Mathematical modeling of the circadian rhythm of key neuroendocrine–immune system players in rheumatoid arthritis: A systems biology approach. Arthritis & Rheumatism 60(9), 2585–2594.

Professor Michael Meyer-Hermann studied physics, mathematics and philosophy in Paris and Frankfurt am Main. The subject of his PhD thesis in theoretical elementary particle physics was quantum chromodynamics. He then headed working groups on the dynamics and spatial organisation of complex biological systems in Dresden, Oxford (UK), Frankfurt am Main and, latterly, at the TU Braunschweig and the Helmholtz Centre for Infection Research. In the course of this work he has specialised in the immune system, cancer research, diabetes, infection research and neuroimmune interaction. He brought a close connection with experimental data with him from his days in elementary particle physics and applied it to mathematical biology as the basis of his work.
Prof. Meyer-Hermann heads the Systems Immunology Department at the HZI and is a BRICS Executive Board member.

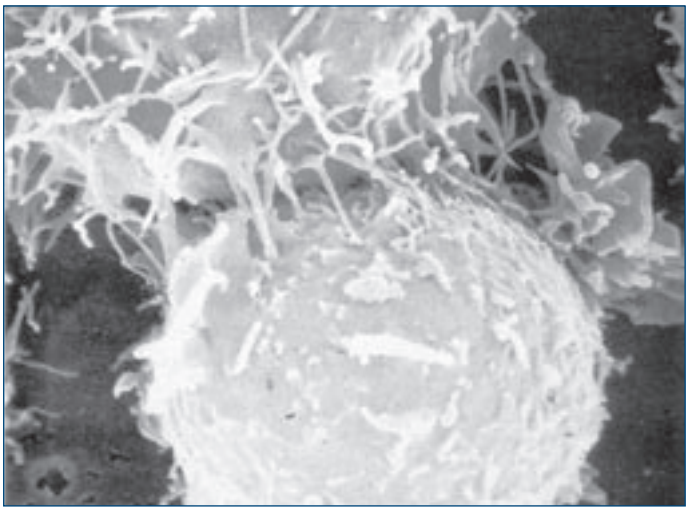
Photo: UFZ

USING THE IMMUNE SYSTEM TO FIGHT CANCER:

Profile of the HELMHOLTZ ALLIANCE ON IMMUNOTHERAPY OF CANCER

Despite all medical progress, cancer continues to be one of the most frequent causes of death. Therefore, in addition to further development of the three classic therapies – surgery, radiotherapy and chemotherapy – the development of new strategies to fight cancer is one of the greatest challenges in health research. Immunotherapy is a novel therapeutic concept specifically using the human immune system to eliminate cancer cells. Many research facilities need to collaborate in order to make successes in this field available to patients. The Helmholtz Alliance on Immunotherapy of Cancer, founded in 2008, unites leading immunologists from four Helmholtz centres and six associated university hospitals. In all, the Alliance builds on the expertise of 21 project leaders.

The Alliance has set itself the target of building a bridge between basic immunology and research using animal models on the one hand, and clinical studies on the other. For this purpose, a budget of EUR 18 million from the Helmholtz Association's Initiative and Networking Fund is at its disposal until the end of 2012.



Dendritic cell, above, with a T-cell, below (Image: DKFZ Heidelberg).

The network focuses on therapies for skin cancer (melanoma), hepatitis and liver tumours as well as for leukaemia and lymphomas. This selection of cancers was chosen as being exemplary for different types of tumour diseases. Liver carcinomas are often associated with a viral infection, which is why specific viral antigens on the tumour cells are suitable target structures for immunotherapy. Leukaemia cells are especially easy to access and therefore suitable for immunotherapeutic treatment. In the Alliance melanomas serve as a model of solid, non-viral tumours and, therefore, cover the type of tumour that is by far the most frequent.

In addition to defending the body from attacks by viruses or bacteria, the immune system also has excellent weapons for fighting tumour cells. The Helmholtz Alliance on Immunotherapy of Cancer would like to harness this potential and help the body to recognise tumour cells.

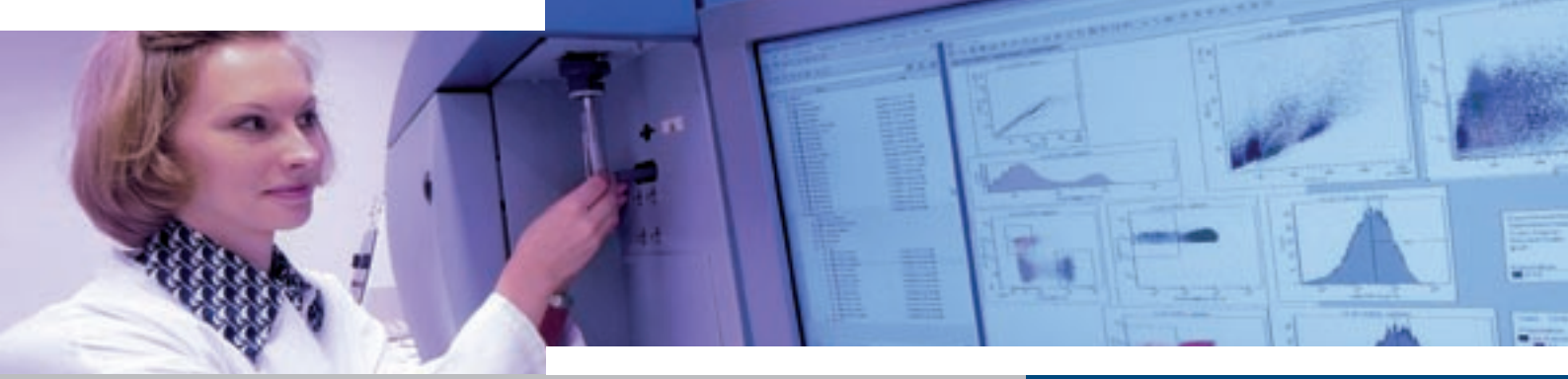
Antibodies and T-cell receptors are highly sensitive proteins that can identify exogenous target structures amongst millions of others. Both antibodies and T-cell receptors can trigger programmed cell death (apoptosis) in damaged cells. Antibodies mark their targets for destruction. T-killer lymphocytes directly kill the target cells identified by their T-cell receptors. To be able to strike, however, both the B-cells that produce antibodies and the T-killer cells need assistance. T-lymphocytes, for example, do not simply identify their targets as they appear in the body. Specialised cells such as dendritic cells must first draw their attention to them. These cells first break the target structure down into its component parts and then present them to the T-cells (antigen presentation).

Therapeutic possibilities arise in programmed cell death, in antibody and T-cell response and in antigen presentation. The Alliance's therapeutic platforms are derived from all of these topics:

- Resensitising cancer cells against programmed cell death
- Novel therapeutic antibodies
- Vaccination strategies against cancer
- Adoptive T-cell therapy

The networking and bundling of forces is the precondition for developing future-oriented immune therapy strategies. T-cell therapy is one such case. Alliance scientists played a leading role in developing adoptive immune therapy, making use of the fact that a donor's T-cells can destroy a patient's leukaemia cells.

A serious problem with adoptive T-cell therapy is, however, that the killer cells also attack normal body tissue. That is why today the challenge is to transfer only T-cells of a certain specificity. This can happen by selecting a donor's killer cells or, in an even more targeted way, by the genetic programming of the patient's own T-cells to recognise and respond to certain tumour features.



Monitoring immune response by analysing cell populations (Photo: HZI, Braunschweig).

HELMHOLTZ ASSOCIATION

Alliance on Immunotherapy of Cancer

Transferring immune cells can be used to fight cancer (Photo: Heidelberg University Hospital).

Different Alliance groups – at the Max Delbrück Center (MDC) and the Charité in Berlin, at the Helmholtz Zentrum München and the Technische Universität München and at the National Center for Tumor Diseases (NCT) in Heidelberg – are engaged in isolating suitable T-cell receptors from T-cells that target tumours. The MDC has special expertise in the cloning of coding DNA sequences and in the development of optimised vehicles for transferring them in human cells. The NCT contributes its outstanding expertise in the development of gene therapy instruments and in assessing the risks involved in the process. This example shows how scientific excellence is bundled in the Alliance and aims at a common objective.

The Alliance's Clinical Studies programme assures to fund the most advanced projects with budgets of their own and thereby to embark on initial therapy trials. On some platforms, such as the vaccination platform, projects are so advanced that clinical studies have already begun.

In every kind of immunotherapy it is not only the effect on tumour growth but also the effect on each individual patient's immune system that must be painstakingly monitored. In developing new therapies it is important to identify and rule out dangerous effects. This observation of the immune system and the success of therapy is known as immune monitoring. Immune monitoring is a general platform in which all of the Alliance sites take part. Networking is especially important in this context. The methods used are standardised within the Alliance and reviewed and adapted in exchange with international consortia.

Vocational and in-service training of young scientists and clinicians is of crucial importance for the success of immune therapy treatment strategies. That is why the Alliance has established a scholarship and exchange programme and runs an extensive programme of education events.

PARTICIPATING PARTNERS:

Helmholtz Centres

- German Cancer Research Center, DKFZ, Heidelberg (Coordinator)
- Max Delbrück Center for Molecular Medicine, MDC, Berlin-Buch
- Helmholtz Zentrum München, German Research Center for Environmental Health
- Helmholtz Center for Infection Research, HZI, Braunschweig

Associated University Hospitals

- Heidelberg University Hospital
- Mannheim University Hospital
- Charité University Hospital Berlin
- Klinikum rechts der Isar, Technische Universität München
- Ludwig-Maximilians-Universität Munich Hospital
- Hannover Medical School (MHH)

The Steering Committee

- Prof. Dr. Peter H. Krammer, DKFZ, Heidelberg (Scientific Coordinator)
- Prof. Dr. Martin Lipp, MDC, Berlin-Buch
- Prof. Dr. Michael Manns, Hannover Medical School, Hannover
- Prof. Dr. Dolores Schendel, Helmholtz Zentrum München
- Prof. Dr. Christof von Kalle, DKFZ and National Center for Tumor Diseases, NCT, Heidelberg

the *escherichia coli* bacterium

A model organism for systems biology

by Katja Bettenbrock, Knut Jahreis, Andreas Kremling, Michael Pfaff, Ursula Rinas, Stefan Schuster and Reinhard Guthke

Escherichia coli, or *E. coli* for short, is a microorganism that is highly suitable for systems biology research. A great deal is known about it. There is a wealth of data and practical applications, and *E. coli* is of great significance to humans in many respects. For example, because it proliferates quickly it is a vital component of intestinal flora, preventing the spread of many harmful microorganisms in the intestines.

On the other hand, pathogenic variants, i.e. those that trigger disease, of *E. coli* also exist. One well-known example is EHEC, which attracted much media attention in spring 2011.

Figure 1: Main focuses of research in the "Dynamics and regulation of the metabolic balance in *Escherichia coli*" FORSYS partner project

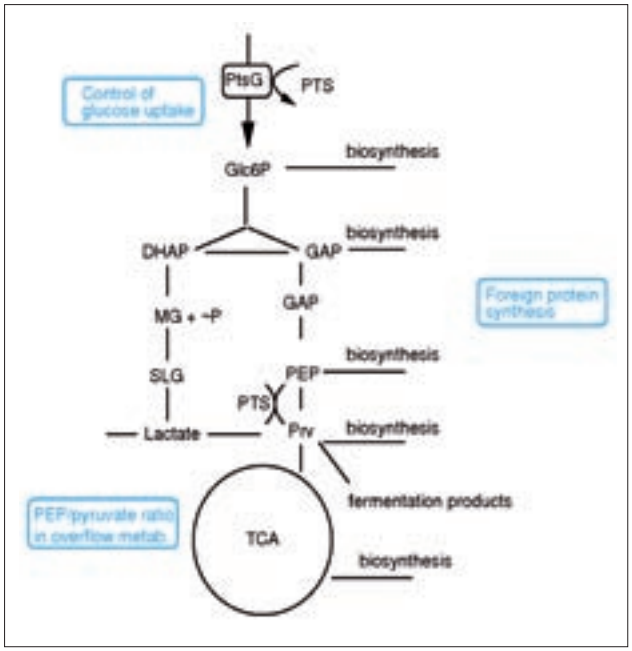


Chart: K. Kremling

E. coli – a workhorse for biotechnology

The bacterium is also significant outside human beings. It is now used, for instance, in the manufacture of an increasing number of important drugs, known as biopharmaceuticals. Following the selective alteration of its genetic information, *E. coli* can be used to produce therapeutic proteins such as insulin. Of 144 genetically engineered drugs, also known as biologics, currently licensed in Germany, 44 are produced with the aid of *E. coli* (www.vfa.de/gentech). Biopharmaceuticals now account for 16% of pharmaceuticals sales in Germany and the proportion is growing.

An obvious goal for scientific and technical efforts relating to biotechnological processes with this kind of economic relevance is to maximise productivity, i.e. the yield from the target protein in terms of process duration and reactor quantity. However, along the path to this goal there are obstacles to overcome.

One such obstacle is the overflow metabolism, whereby unwanted by-products such as acetic acid are formed. However, the term "unwanted" is relative. In another model organism, baker's or brewer's yeast, ethanol, is the by-product, and it is far from unwanted. We already know a great deal about the mechanisms through which these by-products are formed, but not enough to be able to suppress these unwanted processes in *E. coli*. Overflow metabolism is linked to the high uptake rate of carbohydrates and a high glycolysis rate, both of which are necessary for high productivity. The critical controls for the flux of matter are set at the pyruvate node and controlled via gene-regulating networks.

New target genes discovered for an important regulator at the pyruvate node

A three-year research project forming part of the German Federal Ministry of Education and Research's FORSYS partner programme was therefore geared towards understanding and mathematically modelling the regulation of the flux of matter at the pyruvate node in connection with the uptake of carbohydrates and its significance for the synthesis of foreign proteins (Fig. 1) (www.forsys.hki-jena.de).

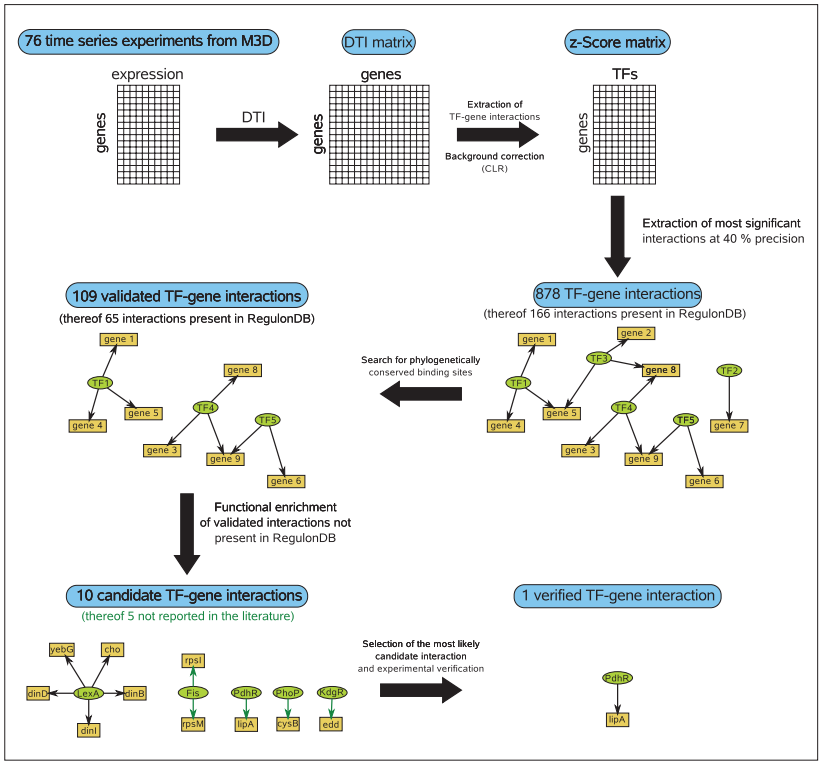
The FORSYS partner project group: Left to right: Christoph Kaleta, Michael Pfaff, Markus Nees, Stefan Schuster, Wolfgang Schmidt-Heck, Frank Wessely, Anna Göhler, Anne Kosfeld, Ursula Rinas, Dominik Driesch, Katja Bettenbrock, Knut Jahreis, Andreas Kremling, Zhaopeng Li, Öznur Kökpinar and Reinhard Guthke (Photo: C. Kaleta).

Genome-wide analysis (Fig. 2) led to a particularly interesting transcription factor called pyruvate dehydrogenase repressor (PdhR) for which, as a result of bioinformatic analysis at the Friedrich Schiller University Jena (Christoph Kaleta, Stefan Schuster) and the Hans Knöll Institute in Jena (Swetlana Friedel, Reinhard Guthke), it was possible to predict new target genes on the basis of gene expression and promoter sequence data. The first prediction was right on target. Anna Göhler and Knut Jahreis at the University of Osnabrück were able to validate in the laboratory the predicted regulation of lipoate synthase (LipA) by PdhR (Kaleta *et al.*, 2010). Further bioinformatic and experimental investigation of the PdhR regulon led to the recent discovery of further targets of the PdhR transcription factor. So thanks to interdisciplinary cooperation between four FORSYS partner project groups across the whole scope of the research cycle, from data analysis via modelling through to experiment planning, the conduct of tests and validation, new gene regulation connections were found. This result would not have been possible without a systems biology approach.

Optimal strategies of metabolism regulation found

In Stefan Schuster's Department of Bioinformatics, Christoph Kaleta and colleagues not only examined individual metabolic pathways and their regulators, but also searched throughout the genome for patterns in the regulation of the metabolic pathways of *E. coli.* This was done applying the elementary flux patterns method recently developed by Kaleta. The researchers noticed that in some reaction pathways all enzymes are transcriptionally regulated throughout, while in others only the first and last steps are subject to a control (Wessely *et al.*, 2011). These peculiarities were explained as a result of evolution: *E. coli* on the one hand minimises the energy costs of protein synthesis, while

Figure 2:



Bioinformatics work schedule that led to the discovery of new gene-regulating interactions in *E. coli* (Chart: Kaleta *et al.*, 2010).

on the other it has to adapt quickly to changing environmental conditions. This explanation was supported by evidence that in reaction pathways whose enzymes are laborious to synthesise because of their great overall protein mass, all reaction steps are transcriptionally regulated throughout so as to avoid unnecessary protein synthesis. However, when catalysis in a reaction pathway is by enzymes with a low overall protein mass, only a few reaction steps are regulated. In cooperation with Martin Bartl and Pu Li of Ilmenau University of Technology, it was possible to show the optimal nature of this pattern of regulation.

Continuing the traditional lines of systems biology in Germany

The research project is continuing along the traditional lines of early systems biology in Germany, laid down by the schools of J. Lengeler (Osnabrück; project partners: Knut Jahreis, Anne Kosfeld, Anna Göhler), E.D. Gilles (Stuttgart, Magdeburg; project partners: Katja Bettenbrock, Andreas Kremling, Markus Nees) and R. Heinrich (Berlin; project partners: Stefan Schuster, Christoph Kaleta, Frank Wessely, Jena). Over the past decade, researchers at the Max Planck Institute for the Dynamics of Complex Technical Systems in Magdeburg, together with colleagues at the University of Osnabrück, have developed complex mathematical models of carbohydrate absorption in *E. coli.* The origins of systems biology at the Hans Knöll Institute (HKI Jena; project partners: Reinhard Guthke, Wolfgang Schmidt-Heck) go back 40 years (Bergter 1972).

The research on metabolic balance in *E. coli* in high-cell-density fermentations brought together colleagues from HKI (then ZIMET Jena) and from the Helmholtz Centre for Infection Biology (HZI, then GBF Braunschweig) as far back as 25 years ago, before Germany's reunification (Deckwer *et al.*, 1990). Project work at the HZI (Ursula Rinas, Öznur Kökpinar, Zhaopeng Li) was and still is geared to the molecular biology foundations for optimising the production of human proteins (e.g. fibroblast growth factor hFGF-2) in *E. coli.* Here, researchers collect transcriptome and proteome data in the stress conditions of external protein synthesis, and analyse and interpret it jointly with the project partners HKI (Wolfgang Schmidt-Heck) and BioControl Jena GmbH (Dominik Driesch, Michael Pfaff).

Systems Biology for Industry workshop in Jena

In autumn 2010, the research project findings were presented at an international workshop in Jena on Systems Biology for Industry: Dynamics and Regulation of the Metabolic Balance in *E. coli.* In addition to the six research units involved in the project, other experts from Berlin, Birmingham, Delft, Jena, Stuttgart and Vienna presented their results.

A representative of the biotech industry, Guido Seidel of Wacker Biotech GmbH, Jena, spoke at the start of the conference. He gave a presentation on ***E. coli se****cretion* **tec***hnology* (ESETEC®) in connection with high-cell-density fermentation (DESETEC®) on the basis of producing antibody fragments and other target-binding proteins. This biotech company and BioControl Jena GmbH have their roots in HKI. Both demonstrated impressively how basic research can yield economic benefits in the long term.

Karin Lemuth (Stuttgart) presented findings from the systems biology school of Matthias Reuss in an impressive talk about the genome-wide analysis and modelling of the *E. coli* transcription and metabolism in glucose-limited fed-batch culture. This made it clear that despite the existence of conclusive, extensive knowledge about *E. coli* under physiological conditions, many questions of relevance to industry about the behaviour of *E. coli* in conditions of recombinant product formation remain open. Systems biology research still has a wide field to explore here.

The research project in brief:

The goal of this consortium project is a full, quantitative understanding of the regulation of carbohydrate uptake and the central metabolism of *Escherichia coli.* To this end, it is modelling the dynamics of metabolic and gene-regulating networks in *E. coli* and exploring the essential structural features of these networks. The central focus of research is on coordination between different metabolic pathways and of overflow metabolism at the pyruvate node.

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robustness of signalling – curse and blessing

Essential for function, problematic for tumour therapy

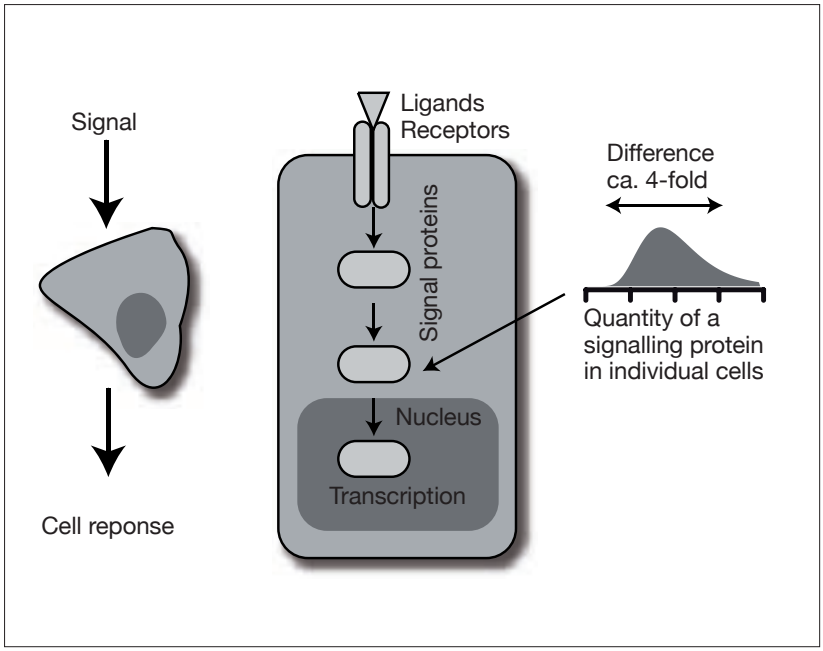
by Nils Blüthgen, Raphaela Fritsche-Guenther, Bertram Klinger, Franziska Witzel, Anja Sieber and Christine Sers

Cells in our body continuously communicate by sending biochemical signals to each other in order to orchestrate collective behaviour and response to perturbations. Cells receive these signals via surface receptors and then process them in a complex biochemical network, the intracellular signal transduction network. An example of such a coordinated process is wound healing. Here, cell division and cell migration are coordinated through signalling pathways, among them the so-called MAP-kinase pathway. In many diseases, especially in cancer, proteins in this signalling pathway are permanently activated by mutations. This results in uncontrolled growth of cells and may lead to the development of tumours. Within our research group funded by the German Federal Ministry of Education and Research (BMBF) and in collaboration with members of the BMBF-funded ColoNet research consortium, we investigate this part of the complex signalling network using mathematical modelling and quantitative cell biological experiments. The aim of our research is to get a better understanding about how this signalling network functions, and to identify the parts of the signalling network that can be well modulated by modern drugs, and help to design targeted therapies.

Signalling pathways – robust information channels in our cells

Modern live-cell imaging technologies have unveiled that two cells of the same cell type and in the same tissue under the same condition differ substantially. Specifically, the protein composition of cells, which mainly execute of biological function, differs strongly. For example, a cell may harbour a million copies of a particular protein, but its neighbouring cell may have twice as much, or only half that number. Biophysicists refer to this phenomenon as "noise" in the expression of these proteins, as these

Figure 1: Signal processing in human cells



Cells in our body receive many different signals from other cells and respond to these for example with growing coordinately after wounding. Many signals arrive in the form of ligands that are received by receptors that in turn activate intracellular signalling proteins. The signal is then passed on to the nucleus, where transcription factors alter the gene expression response accordingly. The quantity of signalling proteins fluctuates strongly from cell to cell, often by more than a factor of four. This gives rise to the question of how signalling proteins, despite these fluctuations, are able to transmit information reliably.
(Chart: N. Blüthgen).

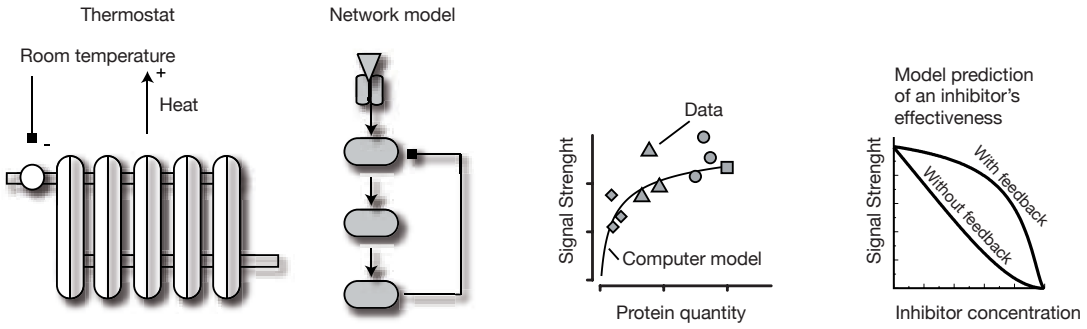


Figure 2: Negative feedback confers robustness
In heating systems thermostats control the heat through a negative feedback. If the temperature is too high, heat input is reduced. If it starts to get cold, heat input is raised again. This maintains an almost constant room temperature. Cellular signalling pathways are equipped with similar feedback mechanisms. By using a mathematical model for a pathway with feedback we predicted that the activity of the signalling pathway would depend only weakly on the amount of signalling proteins, which was validated in experiments. Model simulations for the effect of inhibitors show that if the feedback is intact, high inhibitor concentrations are necessary to block the signal. If the feedback is interrupted, far lower concentrations are sufficient (Chart: N. Blüthgen).

variations occur randomly. This variation in protein quantities seems to be universal, and therefore also the concentration of proteins that constitute the signalling pathways varies strongly. Despite this strong variability, cells can react in a very coordinated way to the many signals they receive (Fig. 1). We therefore asked: How is it possible that, despite the noise in protein quantities, every cell in a group can react meaningfully and uniformly to signals?

We examined to what extend fluctuations in protein quantity effect signal transmission in the MAP-kinase signalling pathway (Fritsche-Guenther *et al.*, 2011). Within the signalling pathway we selectively changed the quantities of individual proteins in cells, and then measured the strength of the signal. To our surprise we found that we could reduce the amount of a central signalling protein (MAP kinase) by around 80% without observing a strong impairment of signal transduction. This proves that the pathway is very robust against changes in protein levels.

Robustness of signalling pathway is a result of negative feedback

In order to explain this robustness, we developed a computer model of the pathway. By running simulations, we found that we can only reproduce such robustness if we add a strong negative feedback loop to the signalling pathway. Such feedbacks are well known in engineered technical systems. One example from our daily live is the thermostat in a heating system (Fig. 2). Such a thermostat allows maintaining nearly constant room temperature, as the heat input is increased upon a drop in room temperature (e.g. because a door is opened). Within the MAP-kinase signalling pathway a similar feedback system buffers changes in protein concentrations. If for example a protein is present in a lower concentration, feedback increases the signal and, consequently, the signal strength remains almost constant.

To identify the molecular basis of the feedback that ensures this strong robustness in the MAP-kinase signalling pathway, we examined various cell lines that showed different mutations (genetic changes) in the MAP-kinase signalling pathway. All cell lines that harbour a feedback that is mediated by a protein called Raf were robust. Cells in which this feedback was disrupted by a Raf mutation were not robust. Thus, without this feedback the cells could not compensate for a reduction in protein concentration, and signalling activity declined. Along with other detailed molecular tests (in cooperation with Tilman Brummer, University of Freiburg) we could pin down the robustness of the MAP-kinase signalling pathway to precisely this feedback mechanism.

Robustness – required for a healthy organism, but counteracting therapy

The robustness of the MAP-kinase signalling pathway and other signalling pathways enables our cells to behave uniformly within a tissue and therefore to react meaningfully to signals. Therefore, robustness is essential for our organism. However, in diseases, especially in cancers, many of these signalling pathways are disrupted. The aim of modern, targeted therapies is to influence precisely those signalling pathways. However, so far these therapies have had only limited success.

We use our mathematical models to simulate how successful low-molecular inhibitors such as those used for targeted therapies can intervene in the MAP-kinase signalling pathway. The calculations show that as long as signalling is controlled by a feedback, large amount of such substances are required to reduce the signal effectively. But our model also shows that in certain cells in which feedback is disrupted due to mutation, much smaller quantities of an inhibitor can clearly reduce the signal strength. This is for example relevant when cells have a mutation in the Raf oncogene, which occurs, for example, in around 10% of all

FORSYS Young Investigator Group: left to right: Anja Sieber, Franziska Witzel, Christine Sers, Nils Blüthgen, Bertram Klinger, Raphaela Fritsche (Photo: Pascal Schulthess).

bowel cancer patients and around 60% of all patients with skin cancer. Our calculations therefore predict that in patients with a Raf mutation in the signalling pathway, a targeted therapy has a good chance to work, whereas its success may be limited if the feedback is still intact. We validated this prediction in cell culture experiments.

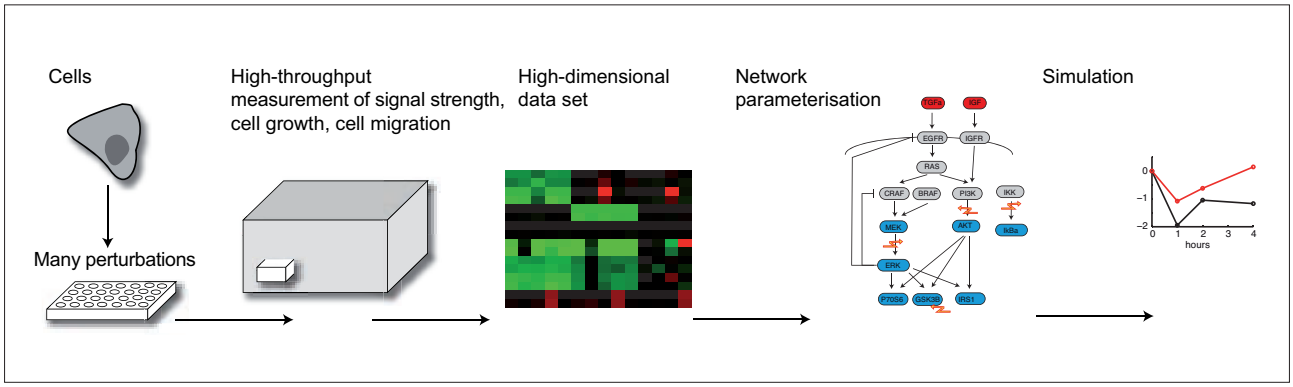
Tracking down the signal

We learned from our study that we need to address the following questions to find optimal points of attack for targeted therapies: How can we now successfully intervene in signalling when signalling pathways are robust? What strategies can be applied for overcoming such feedback mechanisms? Does the signal find alternative routes when we apply inhibitors? To answer these questions, we need a much better understanding of the signalling network, signal strengths and the large number of feedbacks in the system.

In recent years we have developed a strategy for a large-scale, semi-quantitative characterisation of signalling networks (Fig. 3). The underlying idea is to block or stimulate the signalling network at many points, and subsequently to experimentally measure the flow of the signal in the network. The data resulting from this approach enables us to adjust mathematical models of signal flows. We then use that model to plan new experiments that will help us to further improve the models. We then conduct these experiments and adjust the models iteratively. This approach requires novel efficient methods for measuring signalling activities and novel computer algorithms, which we both develop in our group.

The resulting mathematical model can then be used to investigate whether certain parts of the signalling network are more suitable for intervention with low-molecular inhibitors. We can also use the model to dissect the effects of a specific inhibitor on the rest of the signalling network. If we apply our approach to a variety of different cell lines, we can characterise the differences in the way the signalling network behaves in different cells and lay the foundation for understanding why certain inhibitors work very well in some cells and not in others.

Figure 3: Experimental approach for the large-scale characterisation of the signal network



Cells are stimulated in multi-well plates and individual signalling pathways are selectively inhibited. The signalling network status is measured with the help of modern, high-throughput platforms and mapped onto a computer. Network models integrate the data and make it possible to simulate the behaviour of the signalling network, e.g. after inserting a specific inhibitor (Chart: N. Blüthgen).

Demography of cells – the effect of signals on cell growth

The aim of any targeted therapy is to change pathologically disrupted signalling networks in such a way that the cell behaves differently: Either to reprogram it to become a normal cell, so that it does not continue growing after inhibitors are added. Or to selectively kill cells that are abnormal. In order to record these changes in the cell's behaviour, we use a quantitative measuring method that enables us to measure the growth of cells in a culture dish "online" during the experiment. An ongoing "population census" of the cells takes place over several days and we measure directly if and when a signal or inhibitor changes the growth of the cells. Here, again mathematical models are instrumental to analyse the data. Interestingly, models developed in the nineteenth century to measure the growth of animal populations can be applied. We will use and expand these and other methods in future to quantitatively analyse how certain interventions in the signalling pathway exert an impact on the behaviour of cells.

The research project in brief:

FORSYS Young Investigator Group:

Scientists in this BMBF-funded research group are investigating which mechanisms in the intracellular signalling network are responsible for robust signal transmission and hamper targeted inhibition. To this end, they conduct quantitative investigations of selected signalling processes and construct mathematical models of the underlying processes. In close collaboration with the ColoNet research consortium (coordinator: Dr. Christine Sers, Charité Berlin) they explore the extent to which the mechanisms identified are also responsible for resistance to targeted colon cancer therapies.

Participating institutions:

Charité – University Hospital Berlin, Institute of Pathology and Institute of Theoretical Biology

Project leader:

Nils Blüthgen

Reference:

Fritsche-Guenther R., Witzel F., Sieber A., Herr R., Schmidt N., Braun S., Brummer T., Sers C., Blüthgen N. (2011). Strong negative feedback from Erk to Raf confers robustness to MAPK signalling. Mol Syst Biol, 7: 489.

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http://www.sys-bio.net/

long-term view required

The necessity of developing and maintaining databases and standards

by Jürgen Eils, Ursula Kummer and Wolfgang Müller

Systems biology research integrates quantitative, experimental work with mathematical modelling. Therefore, software that enables this integration is a central part of systems biology. However, scientists do not usually work with just one software program, but several software tools that provide different methods of analysis. To ensure that models created with a specific software programme are well understandable and do not have to be rewritten permanently in different formats, efforts were made early to agree on common standards for data exchange.

This gave rise to the SBML (Hucka *et al.*, 2003) and CellML (Lloyd *et al.,* 2004) data standards for the exchange of models. The establishment of these standards has proved its worth for systems biology in other respects, too. For example, it is in the nature of systems biology research to be extremely data-intensive. The integration of knowledge across specific systems necessitates integration of various data sources. In addition, it is essential to make the knowledge gained, whether it is in the form of experimental data or models, accessible within the participating consortiums in particular and to the scientific community in general. Databases have therefore become an important, integral component of systems biology, and standards must be developed for the exchange of types of data. It would make sense for these to be identical to those used by the software tools for modelling.

In addition to the SBML and CellML, standards for experimental data of a diverse nature are needed as well. Standard exchange formats for microarray experiments, MAGE-ML and MAGE-TAB, already exist. Efforts have been also spent to standardise the minimum information that should be available about certain types of data to guarantee reproducibility and reusability. In modelling, this is MIRIAM (Minimal Information Required In the Annotation of Models), in the field of proteomics MIAPE (Minimal Information About Proteomics Experiments) and of transcriptomics MIAME (Minimal Information About Microarray Experiments).

The situation in Germany

In order to discuss the current status quo regarding database solutions used in German systems biology projects and international activities in the development of generally accepted standards, the Project Management Jülich (Projektträger Jülich/PtJ), together with the German Federal Ministry of Education and Research (BMBF) hosted a workshop on "Data Management for Systems Biology and the Life Sciences". It took place on 5th and 6th of May 2011 in Heidelberg and was organised by the authors together with Klaus Mauch (In Silico Biology) and Johannes Schuchardt (Microdiscovery). About 80 participants discussed the current state of the art and further planned activities in the areas of databases and standards. Special existing solutions for managing systems biology data were to be presented and the needs arising from them should be formulated in order to improve the coordination and funding of future national projects. At the workshop, existing applications for administering or analysing a wide variety of systems biology data were presented. Software solutions (SysMOSeek, Virtual Liver Seek, iCHIP and ColoBASE, amongst others) were developed in various systems biology centres for different types of data and assignments. However, in many areas there is no standard laying down minimal information for certain data collected

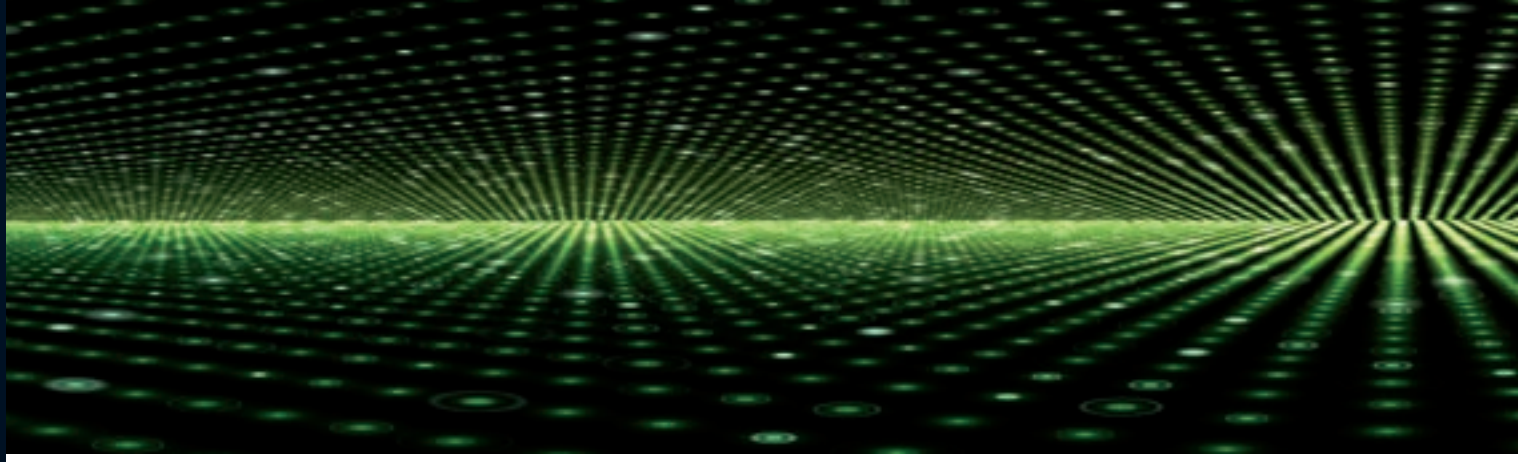

Image: © Thomas R. – Fotolia.com

in diverse systems biology projects or standards enabling the exchange of data. There is currently a lack of such standards in the fields of imaging, next-generation sequencing, proteomics, and protein-protein interaction experiments, amongst others. The participants in the workshop therefore proposed that new standards be implemented in these areas and that existing standards (e.g. PSI, MIRIAM, SBML) be developed further. Funding for both developing and maintaining new standards was criticised as insufficient and the BMBF was advised to increase its funding in this area. All standards currently in use and their details should be collected on a central website. The BMBF should also take the setting up and maintenance of this website into consideration when allocating funding. The diversity and volume of data in the field of systems biology repeatedly confronts data managers with enormous challenges. Curating (producing and checking data quality), in particular, demands both a great deal of time and detailed knowledge of experiments and their context. It was therefore recommended to set up specially developed interfaces to make it easier for potential users of data management systems to integrate data. Even inputting the data is very time-consuming and can hardly be managed by the scientists alone. The workshop participants therefore recommended to employ suitable skilled helpers (students, computer specialists, etc.) for this purpose and to include the costs at the project planning stage already. Data management systems that meet the standards should guarantee the consistency of data and free access to it as soon as it is published. The constructive atmosphere and informative discussions during the workshop are to be resumed in further workshops or training sessions. The positive response from many participants and from the PtJ leads one to hope for early results.

A long-term view requires funding

Certainly, the called-for consistency of data causes a problem for all participants. Data has to be secured and its accessibility checked. A lot of effort is also required for the maintenance of software, including compliance with standards. This is because, as outlined above and discussed in detail at the database workshop, new standards are necessarily being developed and redeveloped continuously within the scientific community in order to enable exchange and store data generated by new methods and models in an appropriate form. Moreover, a program must be adapted to changing circumstances (computer architectures, web servers, databases, program libraries), in order to keep pace with the speed and security demands. New technologies and developments in research and IT call for new strategies to cope with the growing challenges. In the field of next-generation sequencing, for example, such a large volume of data is generated that it is no longer feasible to transfer the entire batch of data to all participating scientists. The use of suitable cloud techniques to provide selected fields of data promises a significant improvement in distribution capacity.

Maintenance and development costs can be argued, but it is hard to get them funded. Funded programmes tend to ask for your own research results in data management, where the main emphasis should actually be put on continuous provision of research results, compliance with standards and the creation of similarities.

Through the PtJ, the BMBF supports data management in the systems biology consortiums that it funds, such as the Virtual Liver and the SysMO consortiums. However, even here, long-term financing to maintain projects' data collections is only partially guaranteed, since no funding can be approved beyond the project duration. Servers, for example, can only be renewed within the term of a project. It will be hard to use cloud technologies because it will be impossible to render account for running costs after the project term. The DFG (German Research Foundation) funds data management as infrastructure within the LIS (Literaturversorgungs- und Informationssysteme / Scientific Library Services and Information Systems) programme. But here, too, getting projects off the ground is the actual purpose of funding. As a result, operators of relevant databases have to guarantee and create ever-new functionality in order to maintain the old functionality. The same applies to software

tools. They are indispensable for research, but there is no funding for maintaining them. The formation and maintenance of an European bioinformatics infrastructure is the goal of the ELIXIR infrastructure project of the European Strategy Forum on Research Infrastructures, ESFRI. The European bioinformatics community hopes that this will lead to less project-tied, longer-term funding of core bioinformatics services, in particular bio-data management. So far, however, no German funding body has signed the Memorandum of Understanding. Moreover, until now ELIXIR includes only a few services that are vital to systems biology. Application sustainability concepts usually aim to offer a service for which payment is required (which is then in competition with services undergoing development that have short-term funding and are therefore free of charge). With the often relatively small number of "customers" that can be reached at present, this undertaking will be hard to implement sustainably, even for large data sources. In addition, this "commercialisation" also has to be administered appropriately and requires a different customer support system, and therefore higher running costs. We hope that the problem can be at least partially solved through a change of thought, for example by means of closer cooperation between the different authors of software solutions which would spread the burden of routine work across more shoulders and simplify mutual reuse of code and data. This also requires a change of mentality that does not disadvantage the continuing maintenance and upgrading of external software solutions that are academically recognised and funded. However, in the final analysis that is no substitute for a long-term view on the part of public funding bodies.

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thinkers, doers and geniuses

The 12th International Conference on Systems Biology – a conference report

by Stefanie Reinberger

Following the invitation from Roland Eils of the German Cancer Research Centre (DKFZ) and Heidelberg University, more than 1,200 scientists came to Mannheim on August 28, 2011, to attend the 12th International Conference on Systems Biology (ICSB). Otmar D. Wiestler, Chairman of the DKFZ and Vice-President of the Helmholtz Association, Simone Schwanitz, Permanent Secretary of the Baden-Württemberg Ministry of Science, Research and the Arts, and host and Conference President Roland Eils officially opened the proceedings. For the next five days an international audience discussed the most current advances in systems biology.

The very large number of ICSB participants was a sign that this still very young research discipline has already come of age. In terms of content, the discipline has plenty to offer, and initial applications are already emerging. Scientists working experimentally are using new methods to produce large volumes of high-quality data, and theoreticians are using ever-more complex mathematical models to chart it on computers.

In light of this, Dieter Münk, Vice President of Storage at the IBM Systems Technology Group, has been enthusiastic about modern systems that not only process large quantities of data but also make intelligent decisions in real time. In early 2011, the Watson computer system played against the reigning human champions of *Jeopardy!* – and won. The computer not only succeeded in retrieving ad hoc information from its multi-terabyte memory, but also formulated grammatically correct questions. Of course, Watson's appearance in a game show was mainly just a gimmick. However, the test run underscores the major advances in computer technology and paves the way for intelligent systems in various areas of life, such as for improving healthcare, for example.

At a press conference during the ICSB, Martin Jetter, Head of Strategy at IBM Corporation, even went as far as saying: "IT is now as important for medicine as the microscope once was." It is, indeed, a fact that only computer-assisted technologies make it possible to fit together the jigsaw pieces of diverse research data to form an overall picture and calculate the most likely courses of events in the system examined. However, looking into the cell, the organ or the overall organism is still a basic prerequisite for successful science.

Modern molecular biology methods for spotting cellular processes are something that Nobel laureate and guest speaker Roger Y. Tsien from San Diego has in his repertoire. Known for his work with the green fluorescent protein (GFP), Tsien will have a lasting influence on molecular and cell biology research. Every biology student is familiar with the fluorophore, a protein obtained from the jellyfish *Aequorea victoria.* Scientists worldwide use it to render cells or their components visible, and thus quantifiable. Tsien and his team de-

Evening lecture at the Sysbio party night.

Conference opening: Ursula Klingmüller (OC Member and Head of Department at the DKFZ), Otmar D. Wiestler (Chairman and Scientific Director of the DKFZ, Vice-President of the Helmholtz Association), Simone Schwanitz (Permanent Secretary, Baden-Württemberg Ministry of Science, Research and the Arts) and Roland Eils (ICSB Conference President, DKFZ and Heidelberg University) (left to right).

A scientific highlight of the conference was the talk given by Roger Y. Tsien (University of California, San Diego, 2008 Nobel Prizewinner in Chemistry).

veloped many derivatives and variants of this protein. Nowadays, a colourful range of fluorescent substances is available, making it possible to distinguish various components in a cell simultaneously.

However, Professor Tsien is now interested in very different molecular biology tools. He is particularly fascinated by so-called miniSOGs (Singlet Oxygen Generators). These are molecules that can be activated by blue light and which are used by scientists to label very precise cellular structures for electron microscopy, as he was able to demonstrate with the help of some impressive images.

However, people who work in systems biology are mainly interested in the dynamics of life processes. Sebastian Maerkl of the Swiss Federal Institute of Technology in Lausanne and his team of engineers and biologists have therefore devised a technology for examining the proteome, the totality of all cellular proteins, of the baker's yeast *Saccharomyces cerevisiae* – and not only in the form of a snapshot. Maerkl used his method for periods ranging from several hours to a day to observe when the yeast cell produces which proteins and at what quantities.

To this end, the Lausanne researchers developed their Microfluidic Platform. At the size of a postage stamp, it is crisscrossed by hundreds of extremely fine channels. The platform is used for examining the fluorescence of yeast cells and simultaneously serves as a culture vessel, guaranteeing constant growth conditions. "Using this system, we succeeded in examining 1,152 strains of a yeast library in an experiment", the researcher said. These strains had changed genetically in such a way that various proteins in the yeast carried the fluorescent GFP tag. The scientists tracked more than 4,000 proteins to establish when and in what quantities they appeared after a specific event, for example the addition of growth factors. "This tool enables us to examine changes in the cellular proteome in real time", Maerkl said.

However, large quantities of data alone do not deliver a conclusive picture of the whole. Rather, the most important thing in systems biology is to abstract life processes skilfully so as to create an overview of general processes.

Markus Covert of Stanford University, California, is exploring, for example, what viruses require from a host cell in order to reproduce successfully. To this end, he infects *E. coli* bacteria with λ phages. Only recently, Covert's team discovered 57 factors in the bacterium that are needed by λ phages in order to reproduce. A close-knit network between host and virus enables the uninvited guest to utilise the cellular activities for its own purpose. Interestingly, many of the key components are important for other viruses, too. Covert believes that it will be possible in future to draw conclusions from this comparatively simple system, which can lead to a better understanding of clinically relevant viral infections as well as to the development of new therapeutic approaches.

Otmar D. Wiestler welcomes international conference participants.

Markus Covert of Stanford University in California.

Engaged in scientific discussion – Thomas Höfer (OC Member and Head of Department at the DKFZ).

Jean Peccoud of the Virginia Bioinformatics Institute in Washington.

Another way to abstract complex processes is to define modules that bring structure to the issue. Large molecular biology networks are often confusing. Instead of failing to see the wood for the trees, Joseph Käs of the University of Leipzig prefers to centre his work around a physically measureable characteristic – the cell elasticity. "Even small changes in the cytoskeleton have major consequences. So we measure effects that cannot be captured at the protein level", said Käs.

As Käs, a physicist, discovered, changes in the elasticity of cells led degenerated cells to reproduce more quickly, to spread rampantly into surrounding tissue and to detach themselves from groups of cells, thereby forming metastases. Measuring cell elasticity could therefore lead to the early detection of cancer of the human mouth and throat area. In addition, initial studies involving breast cancer patients give cause to hope for a new, reliable method of diagnosis for identifying the risk of metastases without having to remove lymph nodes in order to do so.

Incidentally, this was Käs' first visit to the ICSB. He seemed impressed not only by the large number of participants but above all by the conference's very high scientific level. Most colleagues reacted with a kind of curious scepticism to his rather unusual approach to cancer research, he reveals with a grin, adding: "I like that because precisely that gives rise to the best discussions – at least when the exchange of opinions is as frank as here."

Marc Goodfellow of the University of Manchester, UK, is also taking a not entirely traditional approach towards researching a disease mechanism. Together with partners from the University of Berne he is exploring changes in the brains of epilepsy patients. For this, he relies on modelling brain waves. Electroencephalography (EEG) is a standard technique for diagnosing and monitoring this condition. The seizures typical of epilepsy can be ascribed to the spontaneous, synchronous discharge of groups of neurons in the brain. However, the underlying mechanisms are not yet fully understood.

The British scientist aims to use EEG patterns to open up a new perspective on the disease. In his view, it is not enough to concentrate on neurons in the centre of the epileptic fits. Instead, one must look at the whole brain. Using mathematical models, Goodfellow observes large populations of nerve cells that are linked in neuronal networks, taking into account that the cells involved can behave differently.

Goodfellow's models show that regions of the brain in which neurons "fire" with abnormal frequency interact with inconspicuous areas. He concluded that epileptic fits probably happen within a network of neurons with changed behaviour. They may be widely distributed and pass on the pathogenic message to normal cell populations. This disordered activity spreads. Goodfellow believes the crucial task in future epilepsy research will be to examine the spread of abnormal EEG rhythms and to discover how they can be prevented.

Unlike many scientific disciplines, systems biology is not an innovation in evolutionary research, but rather the logical consequence of an existing way of working. "A somewhat systemic view of the way cells and organisms work was typical of this field 20 years ago", says Sandro de Souza of the Ludwig Institute for Cancer Research in São Paulo, Brazil. He wants to know how biological networks develop so as to understand biological systems better. With the help of bioinformatics, he decodes evolutionary mechanisms that create networks in which proteins interact.

Only recently, other scientists showed that this could be ascribed to the multiplication of genes. De Souza's mathematical models, however, point to a different, possibly additional, mechanism called exon shuffling. Exons are DNA segments that code infor-

More than 1,200 international guests attended the ICSB conference in the Congress Center Rosengarten in Mannheim.

Conference President Roland Eils in conversation with guest of honour James Simons (CEO of the Simons Foundation and Non-Executive Director of Renaissance Technologies LLC).

All photos: Yan de Andrés.

mation for proteins. In principle, they are molecular building blocks. When they are rearranged, new proteins with new functions emerge.

"Different domains in a protein interact with each other", de Souza explains. "And if the domains are rearranged by means of exon shuffling, new protein molecules appear that can communicate via these particular domains." According to de Souza, this mechanism could play an important role, especially at key points in the network. It was observed that an especially large number of proteins that emerge following exon shuffling are involved at these points. This indicates that this mechanism may be involved in the development of molecular networks.

One could describe conferences such as the ICSB as one of those points where different (scientific) domains come together in ever new constellations and give rise to new networks and new ideas. Here, scientists from different disciplines meet and industry representatives come into contact with university researchers.

And so at special industry sessions and in the context of an extensive exhibition, numerous companies presented the progress achieved by their systems biology research. Universities, research institutes, and scientific initiatives took the opportunity to present their expertise in the Science Arena, whether with the aim of recruiting talented young scientists or of sponsoring new alliances. Two poster sessions in the permanent poster exhibition offered young scientists in particular the opportunity to present their research. The optics and imaging company Nikon awarded a high-quality camera to the best three poster presentations. The ICSB was supported by a programme of numerous workshops and tutorials in the days before and after the actual conference on the Heidelberg research campus.

Special Events

Film night: BIO:FICTION Science, Art & Film Festival

The screening of award-winning shorts on the subject of synthetic biology from the Vienna Bio:Fiction Film Festival was a particular way of combining art and science. A podium discussion between artists and scientists cast light on the in part provocative depictions of synthetic projects and visions of the future.

How to Get Published

A talk given by Thomas Lemberger, Editor-in-Chief of Molecular Systems Biology, was surely of special interest to junior scientists. With the title "How to Get Published" he explained editorial procedures and the peer review process at the journal, which is part of the Nature Publishing Group. In this talk, he gave future authors valuable tips and hints for publishing in the world of science.

Last-Minute Plenary Session

A few weeks before the conference, employees of the Nature Publishing Group chose, on the basis of current publications, four speakers who were invited to the Last-Minute Plenary Session. This unusual format made the ICSB 2011 especially topical and could well enrich future conferences, too.

SysBio Party Night

To conclude the conference, Hiroaki Kitano, initiator of the ICSB conference series, opened the SysBio Party Night with a lighthearted "night lecture". Graduates, Ph.D. students, and established professors danced and partied into the early hours to the music of a live band and DJ.

news

Nikolaus Rajewsky honoured with Leibniz Prize for setting up new standards in Systems Biology

Nikolaus Rajewsky, Professor of Systems Biology at the MDC and the Charité Berlin and Scientific Head of the Berlin Institute for Medical Systems Biology (BIMSB) has been honoured with Germany's most important research award, the Gottfried Wilhelm Leibniz Prize. The 43-year-old scientist is the youngest of eleven prizewinners coming from different disciplines. The prize is endowed with 2.5 million Euros. The German Research Foundation (DFG) awards this prize to exceptional scientists and academics for outstanding achievement in their respective fields of research.

With great creativity and productivity, Nikolaus Rajewsky's work combines physics and mathematics with systems biology, which examines the regulatory processes in cells or organisms across the genome or proteome. Thus, he has set new standards in systems biology and enriched the life sciences as a whole.

Nikolaus Rajewsky
(Photo: David Ausserhofer/ Copyright: MDC)

His research activities focus mainly on microRNAs, a group of genes discovered only a few years ago. As he has demonstrated experimentally and with the aid of bioinformatics, microRNAs play an important role in the regulation of genes, including those that play a crucial role in the development of diseases. This has profoundly changed the understanding of how genes are regulated and opens up an immense field of research to develop novel therapy approaches.

In addition, Nikolaus Rajewsky and his group have also made important methodological and technological advances by developing, in collaboration with Marc Friedländer, a computer-based method whereby microRNA molecules can be identified. Together with Matthias Selbach he could demonstrate how microRNAs regulate the activity of genes and thus can steer the production of thousands of proteins.

Nikolaus Rajewsky studied mathematics and physics at the University of Cologne, Germany, where he earned his PhD in theoretical physics in 1997. He went to the USA as a post-doctoral fellow at Rutgers University in New Jersey and at Rockefeller University in New York, where he became Research Assistant Professor and, in 2003, Assistant Professor at New York University.

Source: MCD Press Release

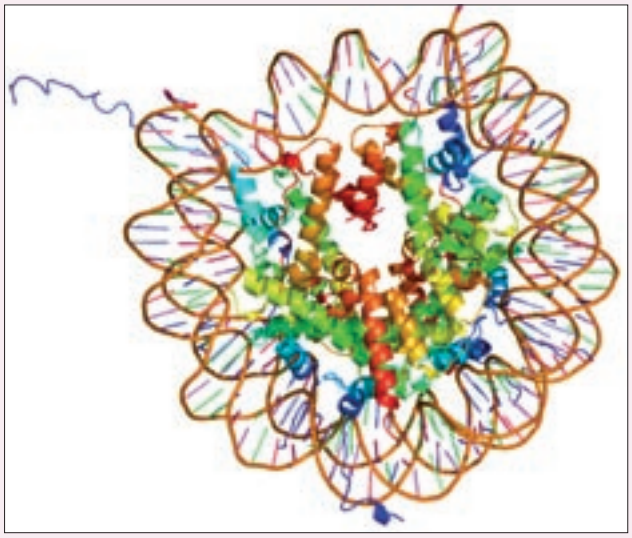
Defects in the packaging of genetic material in malignant brain tumors

Glioblastomas grow extremely aggressively into healthy brain tissue and, moreover, are highly resistant to radiation therapy and chemotherapy. Therefore, they are regarded as the most malignant type of brain tumor. Currently available treatment methods are frequently not effective against this type of cancer.

In order to gain a better understanding of the molecular processes involved in the development of such tumors with a view to develop novel treatment approaches, scientists of the German Cancer Research Center, Heidelberg University Hospital and McGill University in Canada have now deciphered the genetic material of 48 pediatric glioblastomas.

In children with glioblastoma, the scientists have discovered genetic alterations that affect the function of DNA packaging proteins known as histones. DNA molecules wrap around histones proteins that function like coils in the cell nucleus. In a third of cases the histone genes themselves were found altered, in other cases the alterations affected the genes coding for two other proteins that assist in wrapping the DNA around the histone coils. Up until recent years, histones were believed to be little more than DNA packaging material. However, by now it is known that they actively participate

in regulating cell functions. A multitude of chemical tags at specific positions of a histone determine whether or not a gene can be accessed and thus transcribed.



DNA wrapped around histon proteins.
(Image: Emw, Wikimedia Commons)

"The mutations we discovered tend to affect particularly those regions of the histone that regulate gene activity. Therefore, tumor cells with histone mutations have an altered gene activity profile," says Stefan Pfister, pediatrician and molecular biologist at Heidelberg University Hospital. He further explains: "We have discovered the first histone mutation that is implicated in a disease. A single small histone defect can result in major changes in gene activity and, moreover, affect a cell's life span – these two effects together can lead to cancer."

This research project was funded by the German Ministry of Education and Research (BMBF) and German Cancer Aid (Deutsche Krebshilfe e.V.) as part of the funding of the German "PedBrain Tumor" project within the International Cancer Genome Consortium (ICGC; see also page 22).

Original publication: Schwartzentruber *et al.* (2012). Exome sequencing identifies frequent driver mutations in histone H3.3 and ATRX-DAXX in paediatric glioblastoma.
Nature, DOI: 10.1038/nature10833.

Source: DKFZ press release

Kynurenine makes tumours aggressive – Scientists from Heidelberg and Leipzig publishes in *Nature*

Scientists from Heidelberg University Hospital, the German Cancer Research Center in Heidelberg and the Helmholtz Centre for Environmental Research (UFZ) in Leipzig have discovered in a mouse model a new metabolic pathway that makes malignant brain tumours (glioblastoma) more aggressive and weakens the immune system. The newly discovered metabolic pathway, in which the enzyme tryptophan dioxygenase plays a role, catalyses the formation of the kynurenine molecule from the proteinogenic amino acid tryptophan, which is ingested with food. The researchers were especially surprised to find that kynurenine activates the endogenous dioxin receptor, also known as the aryl hydrocarbon receptor (AhR). Previously, scientists had only known that this receptor is activated by various environmental toxins. Activation by the endogenous metabolite leads to a cascade of reactions that ultimately encourage the tumour to grow and to weaken the immune system. High concentrations of kynurenine induce especially aggressive brain tumours. Although kynurenine was discovered in glioblastomas, it can also be detected in other cancerous entities. This discovery is therapeutically highly promising, since using drugs to block this metabolic pathway could be a new, extremely efficient target for cancer treatment. This applies especially to glioblastomas, which are often extremely aggressive and therefore hard to treat. The research project findings were published in the scientific journal *Nature*. Christiane Opitz and Ulrike Litzenburger were awarded the Waltraud Lewenz Prize for this work.

Original publication: Opitz, C.A., Litzenburger, U.M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., Jugold, M., Guillemin, G.J., Miller, C.L., Lutz, C., Radlwimmer, B., Lehmann, I., von Deimling, A., Wick, W. and Platten, M. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature 478, 197-203.

Source: Heidelberg University Hospital press release

Systems Biology – International centres, programmes and initiatives

Digest of university and non-university systems biology activities worldwide

To assist with preparations for specific inter-federal state funding programmes or international research projects, Project Management Jülich has produced a digest of university and non-university systems biology activities worldwide. It juxtaposes international efforts in this innovative research field and brings together existing systems biology centres around the world, their organisation and thematic alignment, along with information on funding.

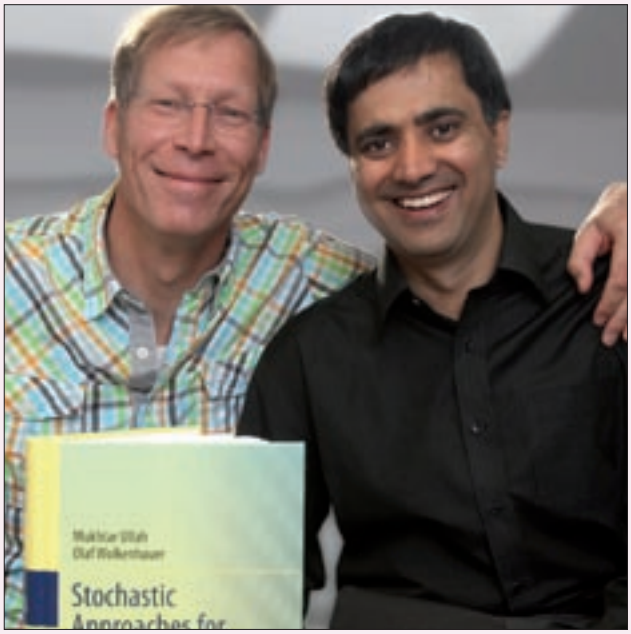
The brochure "Internationale Übersicht der Systembiologie" can be viewed at www.ptj.de/publikationen.

New book published: *Stochastic Approaches for Systems Biology*

In many areas of life, chance events are seen as a nuisance, but in nature chance plays a very important role. It is responsible for the marvellous diversity in nature. However, describing stochastic processes is significant not only on a large scale, but also on a small scale, for example in a cell. The newly published book *Stochastic Approaches for Systems Biology* deals with the mathematical and computer-aided description of chance processes in molecular and cell biology.

There are many approaches to this, and one of the book's main aims is to explain when stochastic approaches in systems biology are advantageous or unavoidable, and how seemingly disparate mathematical concepts are connected. Good notation, i.e. standard notation for very different approaches, plays an important role in this. In order to make the supposedly abstract mathematics easier to digest, the book contains many practical examples, exercises and experiments using computer simulations.

For further information, see:
www.sbi.uni-rostock.de

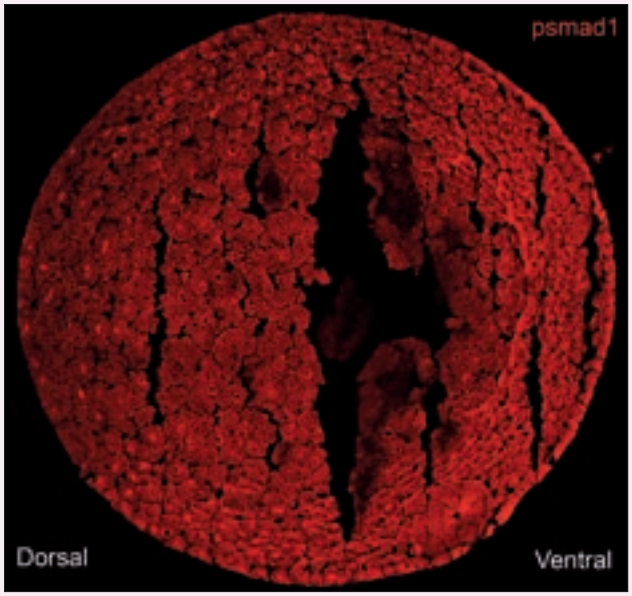


Olaf Wolkenhauer and Mukhtar Ullah are the authors of the book *Stochastic Approaches for Systems Biology* (Springer Verlag, 2011).

Negative feedback regulation generates the dynamic regulation field of morphogenetic proteins in vertebrates

Vertebrates such as human beings are distinguished by the fact that within a few days or weeks they develop a fertilised egg cell first into an embryo and then into an organism with a complex spatial structure and organs. For this, the right numbers of hundreds of cell types have to divide at the "right" place in the embryo, to grow and to contact neighbouring cells.

In their publication, scientists from the German Cancer Research Center in Heidelberg have explored why the signalling cascades of bone morphogenetic genes or proteins (BMP) involved in this morphogenetic process in *Xenopus* embryos always contain feedback inhibitors. By forming BMP gradients in specific regions of the embryo, the BMPs ensure that the morphogenetic process along the dorsal-ventral axis proceeds correctly. A robust interpretation of the BMP gradient across a wide area of concentration is essential for embryonic morphogenesis.



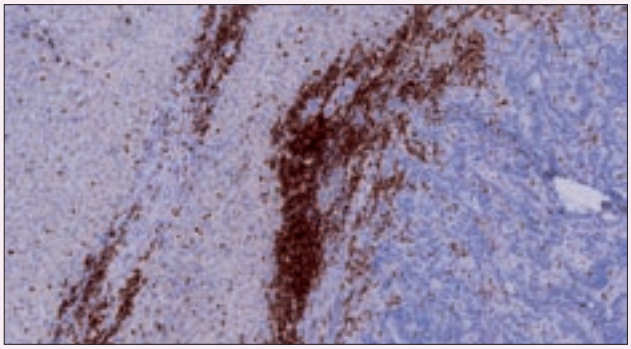
Confocal microscopy of the psmad1 gradient in the gastrula of a *Xenopus* embryo. Cryosections were tinted with psmad1-specific antibodies. This makes the mesoderm surrounding the embryo and a psmad1 gradient visible (Image: Malte Paulsen).

The findings published in PNAS show, with the help of biological experiments on Xenopus embryos and complex modelling approaches, that it is the feedback regulators of the BMP signalling cascade that create the robustness and a large, dynamic regulation and function area. The study also shows that orchestration of the feedback regulators within the framework of a synexpression group optimises robustness and dynamic flexibility. The results therefore helped us to understand the design principles of the BMP signalling cascade, which had long been known, but had not been explained.

Original publication: Paulsen, M., Legewie, S., Eils, R., Karaulanov, E. and Niehrs, C. (2011). Negative feedback in the bone morphogenetic protein 4 (BMP4) synexpression group governs its dynamic signaling range and canalizes development. PNAS 108, 10202-10207.

New marker for the effectiveness of chemotherapy in colorectal cancer

Colorectal cancer is one of the most common forms of cancer. It is the cause of 27,000 deaths in Germany each year. The patient's chances of recovery depend heavily on the malignancy that has probably advanced when the disease is diagnosed. At the same time, the patient's immune system seems to have a critical impact on both the development of the tumour and the success of chemotherapy. Colorectal cancer patients whose tumours have already metastasised into the liver are more likely to benefit from chemotherapy if an increased number of certain immune cells are present on the tumour's margin. Scientists in Dirk Jäger's team at the National Center for Tumor Diseases Heidelberg (NCT) observed this connection during quantitative analysis of liver metastases samples taken from colorectal cancer patients. This specific immune cell density is the patient's immune system's individual response to the tumour and can be used as a prognostic marker for the effectiveness of chemotherapy in the treatment of colorectal cancer patients.



The individual cell density of immune cells on the tumour margin (coloured red) enables conclusions to be drawn as to whether the patient will respond to chemotherapy (Image: Niels Halama & Niels Grabe).

The study was conducted in cooperation with Niels Grabe's systems biology research group at the BioQuant Center. They use an automatic microscopy system that enables precise quantitative mapping of the individual immune response in the tumour samples. And they are currently engaged in joint development of a procedure for identifying this new prognostic marker that can be used in everyday clinical practice.

Original publication: Halama, N., Michel, S., Kloor, M., Zoernig, I., Benner, A., Spille, A., Pommerencke, T., von Knebel, D.M., Folprecht, G., Luber, B., Feyen, N., Martens, U.M., Beckhove, P., Gnjatic, S., Schirmacher, P., Herpel, E., Weitz, J., Grabe, N. and Jaeger, D. (2011). Localization and density of immune cells in the invasive margin of human colorectal cancer liver metastases are prognostic for response to chemotherapy. Cancer Res 71, 5670-5677.

Source: Press release of the National Center for Tumor Diseases, Heidelberg

events

Conference on Systems Biology of Mammalian Cells – SBMC 2012

July 9 – 11, 2012, Leipzig, Germany

In order to present German and international advances in systems biology, this year the Virtual Liver Network will organise the fourth Conference on Systems Biology of Mammalian Cells (SBMC), which will take place from 9 to 11 July 2012 at the Gewandhaus in Leipzig. This international conference will not only bring together eminent international experts from science and industry, but will also address core issues of modern systems biology research with a debate between Sydney Brenner and Denis Noble, two leading international representatives of the life sciences. In addition, the conference programme will once more include the presentation of the MTZ® Award for Medical Systems Biology for the three best doctoral theses in the field of medical systems biology. The SBMC as the most important event of systems biology of mammalian cells previously attracted great international attention in 2006 in Heidelberg, in 2008 in Dresden and in 2010 in Freiburg (www.sbmc06.de, www.sbmc08.de, www.sbmc2010.de). Now, the Virtual Liver network would once more like to seize and further develop the opportunities for exchange of information between medicine, the pharmaceuticals industry and biotechnology.

For further information, registration and submission of abstracts, see:

www.sbmc2012.de

SYSMED 2012 – International Conference in Systems Medicine

September 9 – 13, 2012, Dublin, Ireland

Systems Biology Ireland (SBI) will host international conferences dedicated to clinical involvement in systems medicine, which will be held in Ireland in September 2012 and September 2014. Each conference will have an accompanying workshop, for training early career clinicians and researchers, which will be held in Luxemburg at the LSCB in June 2013 and June 2015. The series will raise awareness of new developments and technologies in this field and exploit opportunities and challenges by focusing the community on medically driven problems. Communication between scientists, clinicians, patients and the healthcare community is critical; all need to understand where they fit in the big picture in order to define their roles and responsibilities. This conference series is open to and welcomes disease focused clinicians and researchers alike. ICSM will take place in the Carton House Hotel, fourteen miles outside Dublin and within very close driving distance of Dublin Airport.

Coordinators:

Conferences: Prof. Walter Kolch, Director, Systems Biology Ireland.
Workshops: Prof. Rudi Balling, Director, Luxemburg Centre of Systems Biomedicine.

Further details:

www.sysmed2012.eu

Bild: SBI, Irland

EMBO Conference Series

From Functional Genomics to Systems Biology

17–20 November 2012

EMBL Advanced Training Centre
Heidelberg, Germany

Confirmed Speakers

Karen Adelman
National Institute of Environmental Health Sciences, USA

Rudolf Aebersold
Swiss Federal Institute of Technology Zurich, Switzerland

Patrick Aloy
Institute for Research in Biomedicine, Spain

Brenda Andrews
University of Toronto, Canada

Jürg Bähler
University College London, UK

Peer Bork
EMBL Heidelberg, Germany

Søren Brunak
Technical University of Denmark and University of Copenhagen, Denmark

Frank Buchholz
Max Planck Institute of Molecular Cell Biology and Genetics, Germany

Wouter de Laat
Hubrecht Institute, The Netherlands

Anne-Claude Gavin
EMBL Heidelberg, Germany

Nevan J. Krogan
University of California, San Francisco, USA

Dana Pe'er
Columbia University, USA

Norbert Perrimon
Howard Hughes Medical Institute, USA

Yitzhak Pilpel
Weizmann Institute of Science, Israel

Frank Pugh
Penn State University, USA

Nikolaus Rajewsky
Max Delbrück Center for Molecular Medicine, Germany

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Kyoto University, Japan

Pamela A. Silver
Harvard Medical School, USA

Athanasios Typas
EMBL Heidelberg, Germany

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RIKEN Center for Developmental Biology, Japan

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Hubrecht Institute, The Netherlands

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Whitehead Institute for Biomedical Research, USA

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Organisers

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EMBL Heidelberg, Germany

Frank Holstege
University Medical Center Utrecht, The Netherlands

Lucas Pelkmans
University of Zurich, Switzerland

ONLINE REGISTRATION ONLY

www.embl.de/training/events/2012/OMX12-01
Registration is on a first-come, first-served basis.
Short talks will be selected from abstracts.

Registration
and abstract deadline is 6 September 2012

Registration fees

Academia	425 EUR
PhD Student	375 EUR
Industry	650 EUR

Contact
European Molecular Biology Laboratory
events@embl.de

Co-funded by the EMBL Corporate Partnership Programme

www.embl.org

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The 13th International Conference on Systems Biology - ICSB 2012

August 19 – 23, 2012, Toronto, Canada

The International Conference on Systems Biology (ICSB) provides an annual authoritative overview of the latest developments in systems biology. ICSB 2012 will be centred on the University of Toronto campus in the heart of beautiful downtown Toronto.

Organisers

The organisers of ICSB 2012 include Charlie Boone, Brenda Andrews, Gary Bader, Cynthia Colby, Tim Hughes, Fritz Roth, Corey Nislow, Sachdev Sidhu, Michael Snyder, Hiroaki Kitano, Edda KIipp, Roy Kishony, Stephen Michnick, Anne-Claude Gavin, Stephan Hohmann, Michael Hucka, and Roland Eils.

Sessions

There will be more than 20 parallel sessions that will involve invited chairs and numerous presentations selected from the abstracts of meeting participants. The sessions will centre on topics involving systems biology approaches to next generation sequencing, genetic networks, computational tools and algorithms, chemical biology, personalised medicine, plant sciences, protein interaction networks, protein engineering, gene expression, modelling and experimental design, and others.

Inspiration

In addition to the science programme, the organisers will offer creative inspiration on other levels. They've planned a number of musical events with international artists, a poster session with a beer tasting from some of the finest Canadian microbreweries, a conference dinner at the picturesque and eco friendly Toronto Brickworks, and much more. ICSB 2012 will close with an optional trip to one of the seven nature wonders of the world – the Niagara Falls.

Registration and more on:

www.icsb2012toronto.com

1. SysPatho Workshop - Systems Biology and Medical Applications

September 11 – 14, 2012, St. Petersburg, Russia

In September 2012 the first SysPatho Workshop on Systems Biology and Medical Applications will be held in Pushkin near St. Petersburg, Russia. The workshop organiser is SysPatho, a scientific initiative funded in the EU's Seventh Framework Programme. The central goal of the 4-day workshop is to promote scientific exchange between European and Russian systems biologists. SysPatho project coordinator Roland Eils: "This meeting will be extraordinarily interesting for Western European participants both in terms of content and because of creating Russian collaborations with protagonists of a research landscape that is traditionally very heavily oriented towards and dominated by mathematical and scientific theory." The workshop programme includes keynote lectures and extensive poster sessions. In addition, special sessions will be used to seek explicit opportunities for new European-Russian cooperative ventures and projects. Workshop coordinator Lars Kaderali: "One main area of the workshop will be dedicated to making new contacts or improving existing ones at the level of junior scientists, so this meeting will surely mark the start of some new, promising cooperative ventures."

For further information and registration (max. 100 participants) see:

http://tu-dresden.de/med/syspatho2012

Photo: St. Petersburg, © pictured by Лунный Ёж

imprint

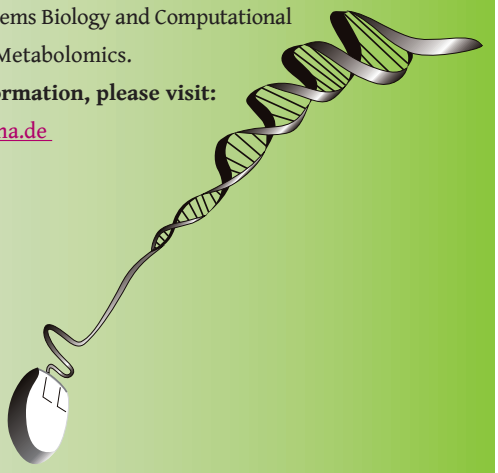
German Conference on Bioinformatics - GCB 2012

September 20 – 22, 2012, Jena, Germany
September 19, 2012 Pre-Conference Workshops

Joining Evolution Networks Algorithms

One of the first German bioinformatics meetings bringing together computer and natural scientists took place in Jena in September 1994. Two years later, in 1996, the series of German Conferences on Bioinformatics (GCB) was started in Leipzig. In 2012, bioinformaticians from Germany and abroad will meet again in Jena on the occasion of GCB 2012. The conference will cover all aspects of Bioinformatics and Systems Biology. It will feature a regular paper, a highlight and a poster track. Regular papers will be published in the conference proceedings in the OpenAccess Series in Informatics of Schloss Dagstuhl. Highlights should describe recently published work to be presented to the conference participants. All contributions will be peer-reviewed. In addition, on September 19, a number of pre-conference workshops will be organized. These will include the topics Systems Biology of Ageing, Organ-oriented Systems Biology, Network Reconstruction and Analysis in Systems Biology, Image-based Systems Biology and Computational Proteomics and Metabolomics.

For further information, please visit:
www.gcb2012-jena.de



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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 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, and the Virtual Liver Network. 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 (BMBF).

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