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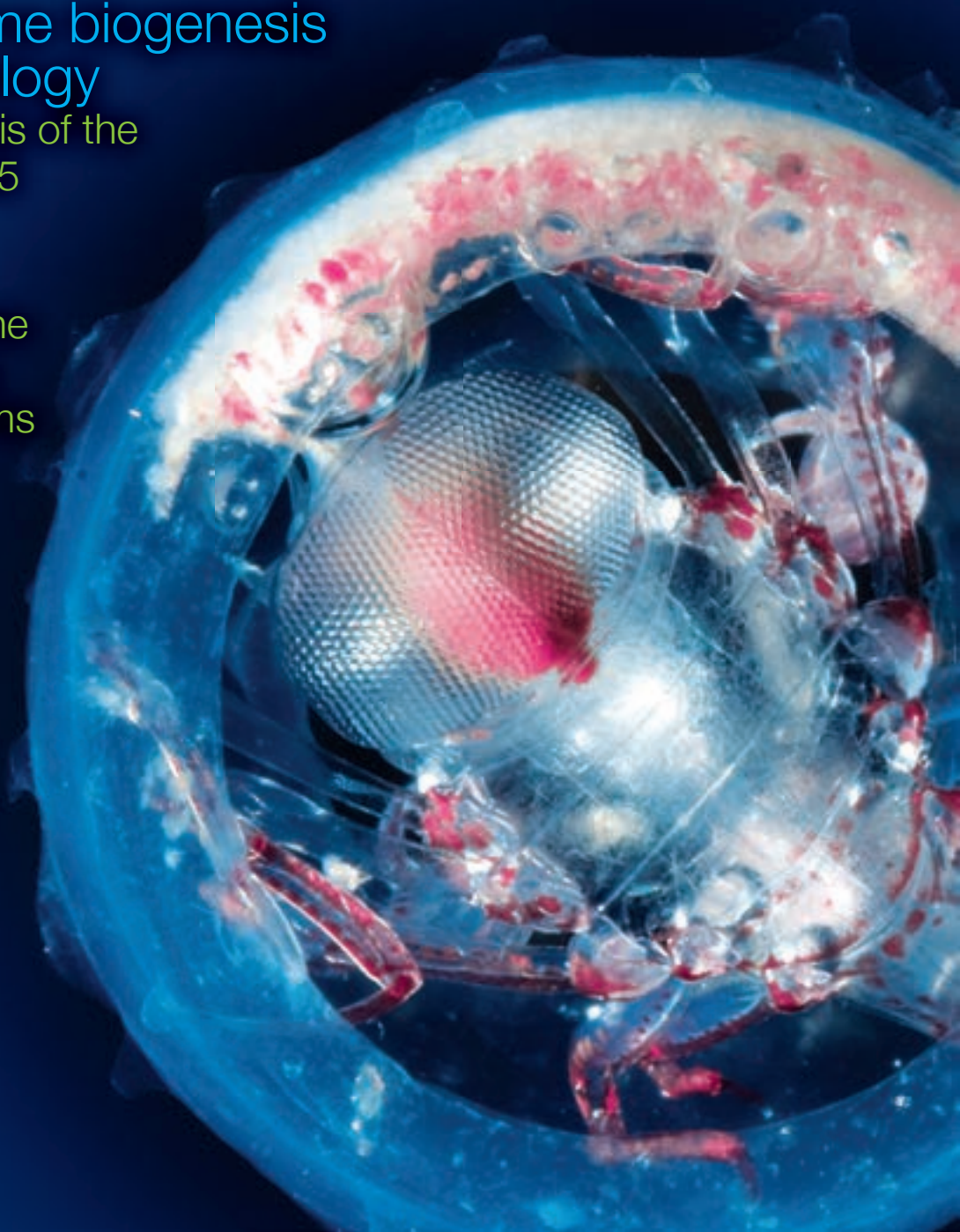

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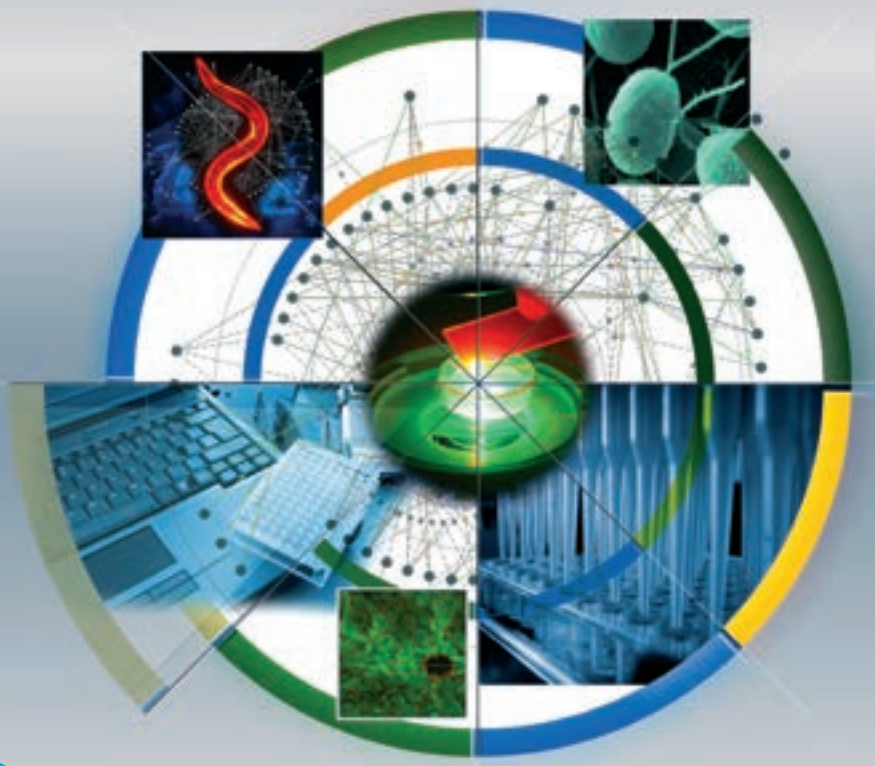

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Systems biology is a young and dynamic discipline that sees the whole picture. As part of the life sciences it builds a bridge between sophisticated laboratory experiments and mathematical modelling, between high-tech data measurements and computer-aided 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.

Cover photo: Amphipod *Pronima sp.* (Amphipoda) (see also article page 61 ff)
Photo: M. Ormestad/Kahikai/Tara Oceans

welcome note

Dear Reader,

The Federal Ministry of Education and Research (BMBF) has been funding Systems Biology in Germany for ten years. More than 430 million Euros have been invested into scientific projects,the promotion of young researchers and infrastructures in this field: centres and institutes have been founded and training and further training opportunities for students created. What is more, international networks and specialist conferences are offering German researchers opportunities for exchanges and worldwide cooperation and giving a decisive impetus to progress in this pioneering field of research.

Systems Biology has experienced an impressive rate of development in recent years. It has become widely established in the molecular life sciences in particular. Today, it is a regular procedure for scientists in health research and biotechnology to use the iterative cycle involving experiments and mathematical modelling when faced with the problem of deciphering complex and dynamic processes.

However, as progress increases so do the demands on Systems Biology. The transition to more application-based research is one particular task facing us over the coming years. By supporting the further development of Systems Medicine, the BMBF is providing an important stimulus for the introduction of this approach in clinical research and practice. The "e-Med – Paving the Way for Systems Medicine" research and funding programme and Germany's participation in the European coordination programme CASyM – Coordinating Action Systems Medicine – are key steps in this direction.

I cordially invite you to continue to actively support the development of Systems Biology and hope that you will find this magazine interesting reading.

Prof. Dr. Johanna Wanka
Federal Minister of Education and Research

foreword

Bad Science

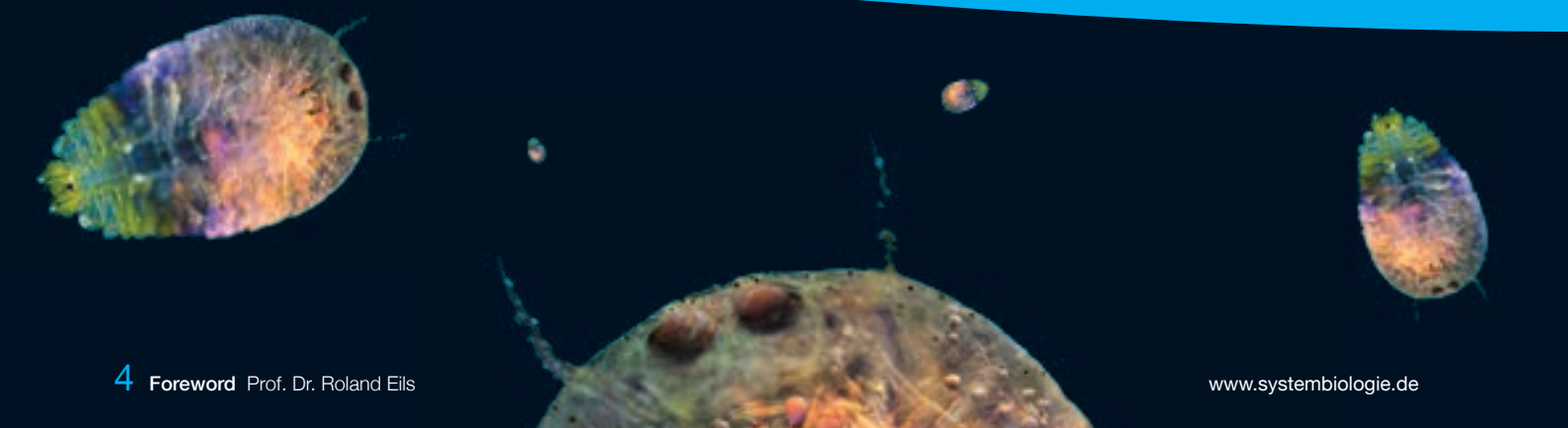
...can be recognized by numerous signs, one of which is the attempt to answer questions that are not actually of interest to anyone – except perhaps to the researcher undertaking it. Good science, on the other hand, sets out to find simple answers to what may be complex questions – answers which shed new light on many things triggering a chain reaction of further scientific studies.

The exciting TARA OCEANS project is an example *par excellence*. This global systems biology project has set itself the ambitious goal of creating a completely new quantitative database for the exploration and study of marine ecology and evolution. The team of researchers headed by Eric Karsenti consequently chose to adopt a systems biology approach.

Their work is based on the idea that systems, forms and functions are closely linked. Systems biologists can therefore no longer be satisfied with identifying the individual components that make up life. They must rather understand how the interaction of these components gives rise to functional entities. The population count of marine organisms and their molecular biological characterization coupled with chemical-physical properties of the seawater and maritime geodata has given rise to a global project in systems biology. It did not take Eric Karsenti long to win the support of numerous mentors and sponsors for this idea, thanks to whom he and his interdisciplinary team of oceanographers, molecular biologists, modelers and bioinformaticians were able to start working right away. TARA OCEANS has quite rightly attracted a lot of media attention in France. Recently, French television has broadcasted several programs on the subject on prime time television in the run-up to Christmas! In Germany, it has taken much longer for the project to gain media interest. Karsenti's article (pp. 61 ff.), however, succeeds to raise enthusiasm as he explains complex relationships and theories in simple words.

Good scientific theories should be explainable to a bar maid, according to Ernest Rutherford (1871-1937). The physicist, born in New Zealand, was awarded the Nobel Prize for Chemistry in 1908 for his pioneering study on the decay of radioactive elements. Is this a call for systems biologists to visit bars more frequently and attempt to explain their work to whoever happens to be behind the bar? Hardly so! But the challenge remains to present the dynamic interactions within a complex research field to the public at large.

Zooplankton Copepod *Sapphirina sp.* (Copepoda) (Photo: C. Sardet and S. Mirshak, plankton chronicles / cnrs, Tara Oceans).

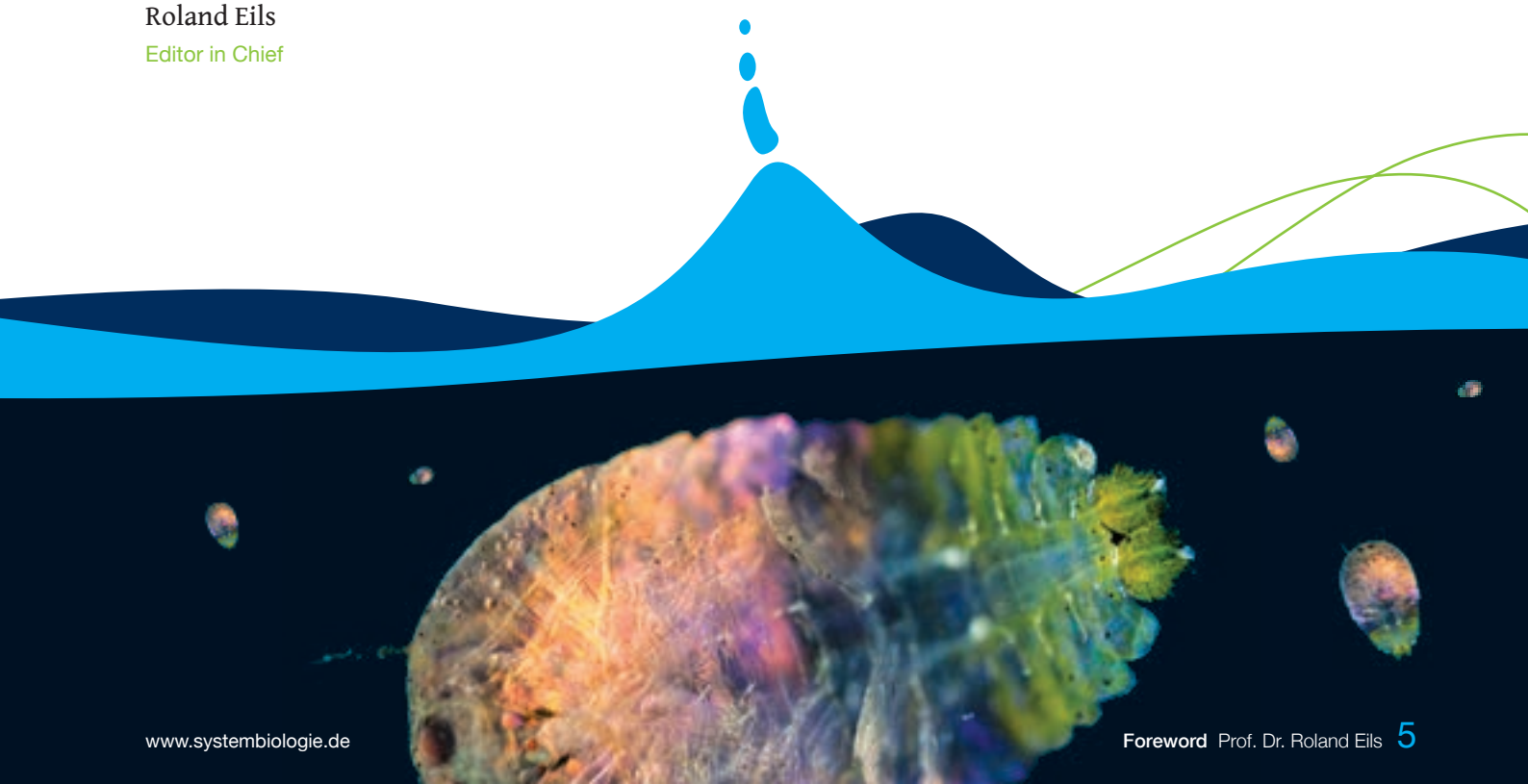


The contributors to this edition of systembiologie.de are known for looking far beyond the horizon of systems biology. They not only have a lot of interesting things to talk about, but know how to express them as well. To gain an overview you need perspective – well formulated by Stefan Artmann who critically explores both the limits and the possibilities of systems biology. From his article, we are pleased to learn that Aristotle and Kant would have been attracted to the field of systems biology (pp. 8 ff.). In turn, I was attracted by Artmann's view of systems biology as a "purposive" ordering system in which "every organ contributes to the survival of the whole organism, but is functional due to the purposive order of the whole organism".

Using a similar analogy, the research community can likewise be viewed as an organism whose individual organs, the research institutes, contribute to the survival of the organism as a whole, just as individual organs would not be viable without the larger organism.

Systems biology is really good science! It provides exciting insights, which in turn set off a chain reaction of questions and answers, enabling us to see things that are supposedly familiar in a new light. There is plenty of this here in this issue of systembiologie.de – certainly enough to whet the appetite for further rounds of questions and answers. In closing let me wish you, dear readers, lots of new insights into familiar and not so familiar fields of research in the systems biology kaleidoscope.

Yours,
Roland Eils
Editor in Chief



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seeing the whole picture requires perspective

Why systems biology should discover its philosophical side

by Stefan Artmann

Systems biology aims to describe organisms as comprehensively as possible in mathematically precise models based on vast quantities of data. There are many philosophical facets to this ambitious goal, ranging from the definition of "system" and the relationship between abstract model and living organism to systems biology's place in the history of science. If systems biologists are now discussing the direction their discipline should take, they would be well advised to invite philosophers to join in the dialogue. After all, there is no shortage of topics to discuss: Are systems "wholenesses"? Can life be understood mathematically? Are we now witnessing the advent of Big Science in biology?

Why Aristotle and Kant would have been fascinated by systems biology

Anyone who sets off in search of the first instance in the Western intellectual tradition of an attempt to explain life itself in rational terms on the basis of empirical observation will sooner or later end up in Ancient Greece, specifically with the philosopher Aristotle (384–322 BCE, fig. 1). Aristotle argued that an object is a living thing if, through a self-regulated process, it develops and retains a characteristic form realizing the purposeful interaction of its organs – such as metabolism and sensory perception. Expressed in today's terms: Aristotle defined a living thing as a self-organizing system made up of functionally differentiated subsystems that together ensure that the system can behave in a certain way.

About 2000 years after Aristotle, one of the most important philosophers of the modern age, Immanuel Kant (1724–1804, fig. 2), described the organism in similar terms. Living things, according to Kant, are objects that are informed by an inner "purposiveness": the organism forms a unit which is self-organizing in that each of its parts is at once both a means and an end for all the others. Thus, each organ contributes to the survival of the whole organism, but is functional thanks only to the purposive organization of the whole organism.

Aristotle and Kant are two philosophers who regarded the property to form systems as a crucial characteristic of all living things. Both would therefore have been fascinated by today's systems biology – and at the same time would have doubted systems biologists' prospects of success.

Systemic or holistic? A call for a clear borderline

Aristotle and Kant would have been troubled by the way systems biology uses the quantitative modeling of physical and chemical processes in an attempt to explain with mathematical precision the systemic quality of all living things. Both regarded any such attempt as fundamentally impossible. Their most important objection was as follows: The systemic unity of a living thing rests on the purposeful engagement of its parts, which has to be described in qualitative terms such as function and purpose. Such terms have no place in the laws of physics and chemistry, however, which are the laws to which the material components of living things are subject and which are concerned solely with quantitative parameters. Kant concluded from this that it was "absurd for human beings ... to hope that perhaps some day another Newton might arise who would explain to us, in terms of natural laws unordered by any intention, how even a mere blade of grass is produced," (Kant, 1987, pp. 282/3). Yet it is precisely to such an explanation of purposiveness based on the "intentionless" laws of nature that systems biology seeks to contribute.

Bearing in mind that philosophers such as Kant and Aristotle would concur with systems biologists that their systemic nature is an essential characteristic of all living things, biologists should make an effort to differentiate clearly between their own definition of system and those that are adduced to

Figure 1: For Aristotle, every living thing is the outcome of a self-regulated developmental process leading to a purposive form.
File: Bild_Aristoteles.jpg (Marble bust of Aristotle. Roman copy after a Greek bronze original by Lysippos, about 330 BC).
Public domain released under: http://commons.wikimedia.org/wiki/File:Aristotle_Altemps_Inv8575.jpg?uselang=de

declare their field of research illusory. This also means that systems biologists must take their leave of concepts such as "wholeness." For in the history of thought, those who present living things as wholenesses and then, logically enough, demand that they be understood holistically, tend to be associated with those philosophical positions which regard the science of biology as fundamentally different from physics and chemistry (Harrington, 1999). The premise of a non-mechanical life force (vitalism) and the insinuation that the structure-building interaction of material processes inside the organism is ordered by factors which owing to the top-down causality of the whole impact on the parts (organicism) are two variations on this theme.

From the philosophical point of view, systems biology is actually an attempt to render holistic doctrines of wholeness superfluous. Systems biologists would therefore do well to bear this in mind if they are to avoid inadvertently becoming complicit in the revival of scientifically misleading philosophies of life.

On the way to a structural science of living systems

The definition of "system" to be used in biology can be developed only in conjunction with other concepts that in the last few decades have become increasingly important to life sciences research. Concepts such as self-organization and information, for example, are indispensable whenever the aim is to make living things scientifically intelligible as functionally complex material systems.

Philosophers with a background in the natural sciences such as Carl Friedrich von Weizsäcker and Bernd-Olaf Küppers use the term "structural sciences" for research programs concerned with the formal definition and empirical application of such concepts (Küppers, 2008, pp. 313 ff). Cybernetics, information and network theory are all examples of structural sciences. Structural sciences use mathematical means to describe abstract relational objects – structures, in other words – such as control circuits, codes, and networks. What drives the structural sciences is not formalization for its own sake, but the application of their formal models to as many different areas of experiential reality as possible. Analogies are drawn between the different areas of application so that, for example, engineers, biologists, and linguists can learn from each other through the medium of information theory.

The starting point for a structural scientific approach in systems biology could be the intuitive notion of a system as a set of related elements in which the relationships between the elements are co-determinants of their behavior, which is what sets the system apart from its environment. To enable us to grasp the dynamic characteristics of organisms in abstract terms, this simple notion of a system can be supplemented by models of self-organization. If the aim is the formal depiction of the internal organization of living things, then it makes sense to use network models. And to help us grasp the functional character of organic processes in mathematical terms, we can avail ourselves of information theory, which regards signals as signifiers on the grounds that their occurrence triggers or inhibits certain processes, indicating that these signals possess a function in the organism (Artmann, 2011).

Combining such structural scientific approaches allows us to define systems biology as that biological discipline which explains the unity of the organism in its environment by showing how, as a complex network in an environment, it uses information to self-organize. Certain aspects of this definition can be given more emphasis than others, depending on the focus of a given research project; if the focus is on the informational aspect, for example, a cell will be viewed primarily as a system that processes meaning (Görlich *et al.*,

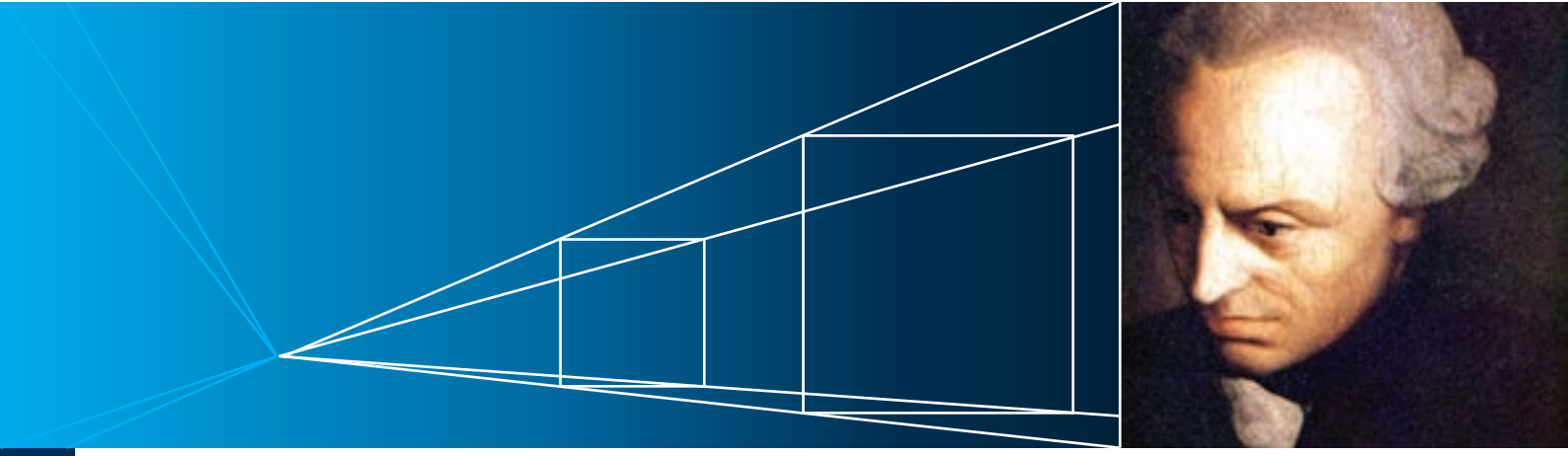

Figure 2: Kant describes organisms as systems in which each part is at once both a means and an end for all the others.
File: Bild_Kant.jpg (Portrait of Immanuel Kant, 18th century).
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2011). Acute awareness of this interaction of structural scientific concepts can help systems biology to meet the empirical and technical challenges that it faces by making better use of the combinational scope of its key concepts.

Quo vadis, systems biology? Per aspera ad astra!

Systems biology is no longer concerned only with positing abstract models of the fundamental characteristics of living things and with drastically simplified realizations of the same, such as those that John von Neumann (1903–1957) attempted in his structural scientific theory of self-reproducing automata. After all, systems biology is at the same time applying such models to real living things. Expressed in philosophical terms, its concern is with the theoretical synthesis of real organisms. This is possible because of the ever greater quantities of data on individual processes in living things which thanks to today's computers we are now able to collect and analyze and which can be used to develop and test mathematical models of the causal structure of ever larger processes in organisms (Küppers, 2008, pp. 412 f.). Synthetic biology builds on this by attempting to create possible organisms in reality.

The goal of systems biology, which is to integrate large quantities of empirical data on individual processes in a given organism in comprehensive mathematical models of the whole organism, inevitably leads to research facilities whose scope far exceeds anything hitherto known in biology. This is the advent of Big Science in biology – even if its organizational forms look different than in physics, which went through the same process more than fifty years ago. This is also a socio sign that systems biology is not a unique phenomenon in the history of modern science, but rather is a discipline that thanks to fruitful, if rather arduous research programs, is now using advances at the theoretical level to explain what is already known at the empirical level and to predict correctly those empirical findings that have yet to be made.

Such a modest view of systems biology's future development may disappoint the exaggerated hopes that have been pinned onto it, but it does at least show that the effort is worthwhile. *Quo vadis,* systems biology? *Per aspera ad astra!* Along stony paths to the stars!

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BioSysNet – the Bavarian Research Network for Molecular Biosystems

Molecular biosystems research – or how life organises itself

by Horst Domdey, Ulrike Kaltenhauser and Claudia Szeibert

Systems biology is one of the most dynamic fields of research today, and also one that is highly future-oriented. The Bavarian Research Network for Molecular Biosystems (BioSysNet) was established in 2011 with the aim of bundling and expanding existing expertise in Bavaria, thereby strengthening this field of research. It offers scientists throughout Bavaria an opportunity to research jointly into current and relevant problems. The network's main emphasis is on interdisciplinary cooperation between different universities and institutes in Bavaria.

The network's structure and mission

Building on the great success of the Bavarian Genome Research Network (BayGene), which was established in 2004, the Bavarian state government set up a new funding programme for research into molecular biosystems. The aim is to bundle the existing expertise of scientists in Bavaria and expand it by recruiting outstanding junior scientists from outside Bavaria, thereby creating ideal conditions for this field of research in the state. Bavaria has made available €18.1 million in subsidies for the scientific expansion of biosystems research, including the Core Center at the Ludwig Maximilians University (LMU) in Munich. It has also allocated €13.65 million for the construction of a new research building at LMU Munich, where the focus will be on 'molecular biosystems'.

With Prof. Dr. Horst Domdey (BioM GmbH) as coordinator, a total of 24 projects are currently funded within the Research Network framework at the Bavarian universities of Erlangen-Nuremberg, Munich (LMU and Technical University), Regensburg and Würzburg.

The initiative has made it possible to bring five excellent, internationally successful young scientists to Bavaria. Each will receive around €1.5 million over the next five years as start-up funding to establish their own, independent junior research groups:

- Dr. Ana Eulalio from the Institute for Molecular Infection Biology at the University of Würzburg is investigating how bacteria manipulate host cell functions to ensure their own survival. She is also examining whether bacteria intervene in the RNA metabolism of host cells and how this benefits the bacterial life cycle. The long-term goal is to develop new therapies to combat bacterial infections.
- At Munich Technical University's Klinikum rechts der Isar, Dr. Olaf Gross is investigating molecular mechanisms of two-stage interleukin (IL) activation. The synthesis of IL-1 is already understood, but its secretion from the cell still raises major questions nonetheless. The primary focus of research is on examining the molecular mechanisms of IL activation and its subsequent secretion. What is known so far is that the release of interleukin is caused by the formation of an inflammasome complex.
- A second junior research group was established at the TU Munich with Dr. Tobias Madl. His research focusses on molecular mechanisms in which intrinsic and unstructured proteins act as key regulators during essential processes in the cell. To examine their function, regulation and malfunction in the case of disease, multidisciplinary approaches using magnetic resonance spectroscopy, small-angle X-ray and neutron scattering and modelling will be combined.
- Translational control is the subject of Dr. Jan Medenbach's scientific work at Regensburg University. The recent discovery of an RNA binding protein that controls the activity of upstream open reading frames (uORFs) has significantly influenced the way in which translational regulation is viewed. The goal of the Regensburg junior research group is to explain at the molecular level how uORFs control protein synthesis and what malfunctions occur in the case of disease.

↗ Dr. Fabiana Perocchi from the LMU Gene Center heads the third junior research group in Munich. Her work focuses on mitochondrial signalling networks and their functions in calcium homeostasis. The aim is to identify molecular machines and mechanisms that control the transmission of information to the mitochondria.

In addition to funding for the new junior research groups, co-financing of 18 previously established research groups at Bavarian universities will safeguard the further development of existing expertise. The intention is to create a fertile environment for close cooperation and sustainable scientific exchange.

Different disciplines – One shared network

To guarantee efficient systems biology research, different disciplines must cooperate. The analysis of complex biological regulation systems is an interdisciplinary endeavour that requires coordinated collaboration between various disciplines such as chemistry, biology, medicine and bioinformatics. New technologies have revolutionised the field of life sciences and initiated a new era in methodology. Along with experimental work, mathematical modelling is now a central component of systems biology.

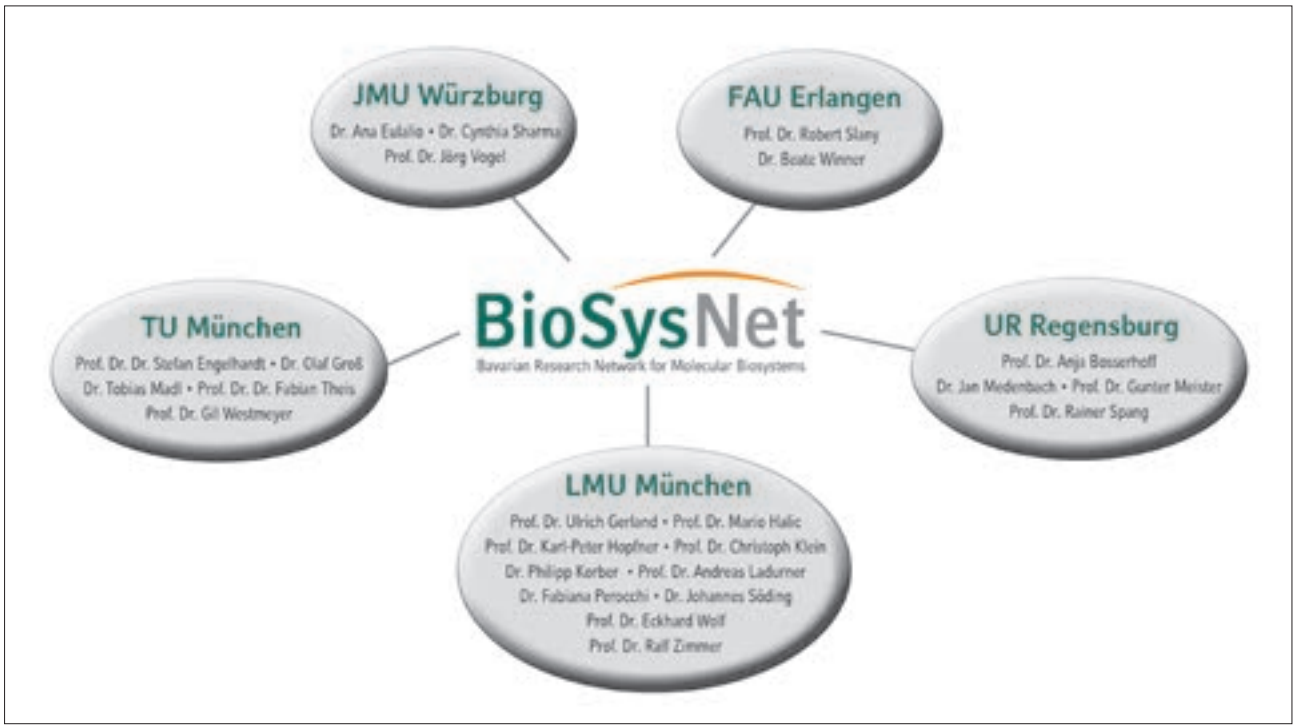
The BioSysNet mission is to enable interdisciplinary networking and close communication between scientists by means of research projects that are funded Bavaria-wide.

BioSysNet is part of the new Bavarian Research Center for Molecular Biosystems

The Bavarian Research Center for Molecular Biosystems headed by Prof. Dr. Patrick Cramer (Director of the Gene Center at the LMU Munich) includes, in addition to the network described above, a new Core Center at the LMU Munich. This builds on the Gene Center, which was established in 1984, and can draw on the capabilities of the Grosshadern/Martinsried campus and the 'Center for Integrated Protein Science' (CIPSM) cluster of excellence. The main focuses of the Core Center are promoting molecular biosystems research, developing high-tech platforms and training scientists who think in interdisciplinary and systemic terms. The Core Center also serves as a coordinating center for both local and Bavaria-wide activities and for linking academia and business. The Core Center will reinforce the Grosshadern/Martinsried campus as an innovative research site and will be buttressed by a new Molecular Biosystems (BioSysM) research building to be constructed by 2015 on the Munich-Grosshadern/Martinsried biomedicine campus.

People all over the world associate Bavaria with a high standard of biotech research in science and industry. This is largely due to the excellent Bavarian universities and non-university research institutes, as well as excellent business conditions that have evolved to this high level with the help of funding from the state of Bavaria. With the Bavarian Research Network for Molecular

Figure 1: Overview of projects funded by the Bavarian Research Network for Molecular Biosystems



Source: BioSysNet

Biosystems, the Bavarian state government is sending a clear signal of its intention to continue resolutely along this path.

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www.biosysnet.de

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Figure 2: Molecular biosystems research – or how life organises itself

Source: BioSysNet

the woman who wants to manipulate hubs of the immune system

Portrait of Ria Baumgrass

by Stefanie Reinberger

"Isn't it nice here?" Ria Baumgrass says, admiring the view through the large glass facade to where the brick buildings of Berlin's venerable Charité Hospital are strung out in the summer sun. In between them stands the modern building of the German Rheumatism Research Center (DRFZ). Its red brick blends into the surroundings while glass and concrete form a distinctive contrast and lend new dynamism. "I think it works very well," the research scientist says with a smile.

She likes working here, in this science-steeped atmosphere where medical tradition and innovative research go hand in hand. "There is a lively exchange of ideas between the different research groups here, and, moreover, with clinicians," she says. The latter, she points out, is especially important, because in the end it is the patient who counts. Ria Baumgrass is researching into T cells. She wants to know how the body's defenders are activated. She hopes her findings will help in understanding the processes that take place in rheumatism and various other autoimmune diseases – and ultimately in laying the foundation for new therapies.

Baumgrass was captivated by research buildings at an early age. "My mother was a chemist and worked in the physiological-chemical institute at Halle University," she says. "My twin brother and I often went there as children and I found it really exciting." She says she still has the special smell, a mixture of chemicals and old masonry, in her nostrils.

The fundament for her later career as a scientist may have been laid at that time, certainly so by the time she graduated from high school. She was determined to study biochemistry in the face of all opposition and advice to the contrary. "There were very few opportunities to study biochemistry in East Germany at that time, and everyone advised me to apply for something else," Baumgrass recalls. However, she applied only for biochemistry and managed to secure one of the coveted places at Halle University.

Perhaps her assertiveness came from her involvement in competitive sport. As a young woman, swimming was her discipline. "I cried many tears in those days, but you learn to persevere and carry on when things are not going too well and you are not enjoying yourself," Baumgrass says. And you find out that after lean periods things get better again, she adds.

Now she believes that to achieve enduring success, the most important thing is to enjoy what you are doing. That is what she told her grown-up children when they were deciding on a career, and it is what she tells her postgraduate students. "It's so nice to see them motivated and developing slowly but surely into self-confident scientists," she says.

Ria Baumgrass is happy to act as their mentor. She is also an enthusiastic teacher – at Potsdam University, where she is a senior lecturer, and at the Autumn School for Immunology, a course that she and some colleagues developed and organise. After all, she herself benefited from motivated teaching as a student. "There were only 15 students per semester, so we were in close contact with our professors," she says.

However, it was a long journey to her field of research and her self-confidence as a scientist. Baumgrass worked in various fields, resigned from a tenured post and was a postdoc in the US before returning to Germany, and to Halle again. There she did research on calcineurin, an enzyme that plays an essential role in activated T cells, and that ultimately paved the way for her work on the immune system.

"I have found my scientific home in immunology," Baumgrass says, explaining why she finds T cells so great. "They are an important part of the adaptive immune response and it is easy to isolate them from blood and work with them," she

Ria Baumgrass heads the Signal Transduction research group at the German Rheumatism Research Center in Berlin (Photo: S. Reinberger).

says. For all its complexity, she says, systems biology is really making its presence felt in this field.

The research scientist gives an example. "We have observed that IL-2 impacts quite differently on different T cell populations depending on the effective dose," she says. High IL-2 doses have a stimulating effect on inflammatory T cells, i.e. the subgroups that provoke a strong immune response but also encourage inflammatory processes such as rheumatism. With low doses of IL-2, it is mainly the regulatory T cell subpopulation that becomes active and multiplies, i.e. the players that prevent inflammatory reactions. "Then there are other sub-groups that react to IL-2 differently again, besides which they all influence each other," Baumgrass says. And of course everything is much more complicated because a large number of messenger substances and transcription factors impact on T cell activation. To obtain an overview of all these processes, you need systems biology.

How the term is defined is not an issue for Ria Baumgrass. "I think both are important – the high throughput methods to enable us to identify all players if possible, and the modelling that enables us to obtain an overview of the most likely scenarios and to plan well-targeted experiments," she says.

She is currently working with her cooperation partners on defining hubs in the activation process of T cells that decide the direction in which the immune response develops. "Once we know them, we may also find ways of influencing excessive defensive reactions in autoimmune diseases and allergic reactions," she says.

Baumgrass takes special pride in the fact that in one case her findings contributed towards an actual improvement in therapy. "I told Margitta Worm, a leading dermatologist at the Charité, about my observations on T cell activation, and she was very interested." That was because doctors use the immunosuppressive drug cyclosporin A to treat severe neurodermatitis. Two approaches exist: either start with a high dose and reduce it gradually, or vice versa. Worm is an advocate of starting with a low dose, and Baumgrass' findings suggested that she was right.

The two women then examined some patients jointly and found that a high dose curbs the excessive defensive reaction but wipes out the entire immune system. A low dose also has a curbing effect, but simultaneously entices regulatory cells from the reserve that reduce the inflammatory reaction further. This therefore helps the immune system to normalise itself to some extent and achieves a better therapeutic outcome with fewer side effects.

"That was very uplifting, because it is rather rare for research findings to be applied so directly," Baumgrass says enthusiastically. The discovery could also benefit patients with conditions such as Crohn's disease or multiple sclerosis. "We are currently testing this on mice, because we need to take the long route again, from laboratory to animal experiments, and maybe one day to a clinical study," she says.

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who turned the clock?

Regulation mechanisms of segmentation in vertebrates

by Hendrik B. Tiedemann, Elida Schneltzer, Stefan Zeiser, Bastian Hoesel, Johannes Beckers, Gerhard K. H. Przemeck and Martin Hrabě de Angelis

Somitogenesis is a process in embryonic development of all vertebrates in which the segmentation of the vertebrate body is laid down. Small balls of cells (somites), from which the vertebrae later emerge, split off from the anterior end of the mesenchymal tissue laterally to the neural tube. This happens with a species-specific periodicity. The molecular principles of this somitogenesis clock are still not fully elucidated. In order to understand these mechanisms better, we developed a computer model that makes it possible to describe these processes by means of differential equations, to solve them numerically for many cells in a tissue, and to visualize the resulting gene expressions patterns vividly.

Segmentation of the presomitic mesoderm

The segmentation of the body's longitudinal axis is a common feature of all vertebrates. The eponymous vertebrae in the spinal column are only one structural element. The nerves and trunk musculature also reflect the periodicity along the longitudinal axis. Segmentation is laid down at an early stage in embryonic development, though not all at once. Rather, the developing embryo 'grows' from head to tail. In the course of this process, a mesenchymal tissue, in which cells are mobile and relatively loosely packed, forms on both sides of the neural tube, the future spinal cord. This presomitic mesoderm (PSM) grows at its posterior part while simultaneously at its anterior end small balls of cells (somites) bud off on both sides of the neural tube with clockwork regularity (Fig. 1). During this process, the PSM grows posteriorly with the same rate at which somites bud off anteriorly. The equilibrium between growth and somite formation ends only when a species-specific number of somites has been reached.

Clock and wavefront model of somitogenesis

This clockwork-like mechanism has fascinated biologists for many years. To explain it, the 'clock and wavefront model' was formulated in 1976 (Cooke & Zeeman, 1976). It postulates that a molecular oscillator causes every cell in the PSM to oscillate between different states while simultaneously a signal with a limited range is emitted from the growing tip of the tail. As soon as the cells in the anterior part of the PSM fall outside the range of this signal, which retreats as the tail tip grows, their current molecular oscillation state is frozen and a boundary is created between the new forming somites and the rest of the PSM. However, this model did not specify the exact molecular and cellular mechanisms that should cause all these processes.

Oscillating genes in the presomitic mesoderm

It was only 15 years ago that the gene *c-hairy1* has been described in the chicken embryo (Palmeirim *et al.*, 1997), showing a periodic behaviour consistent with the model. Its oscillation in every cell is based on the fact that the protein encoded by *c-hairy1* can bind to its own promoter and prevent transcription by RNA polymerase II. However, this blockade does not last indefinitely. The corresponding protein in a mouse (HES7) degrades with a half-life of approximately 20 minutes, as does the associated messenger RNA. This weakens the blockade of the gene and the gene can be read again. The messenger RNA is produced anew and migrates from the cell nucleus into the cytoplasm, where it is translated into the protein, which is then transported back to the nucleus, where it binds to the promoter and blocks it. This game leads to an oscillating gene expression with a period of approximately 120 minutes in a mouse or 90 minutes in a chicken.

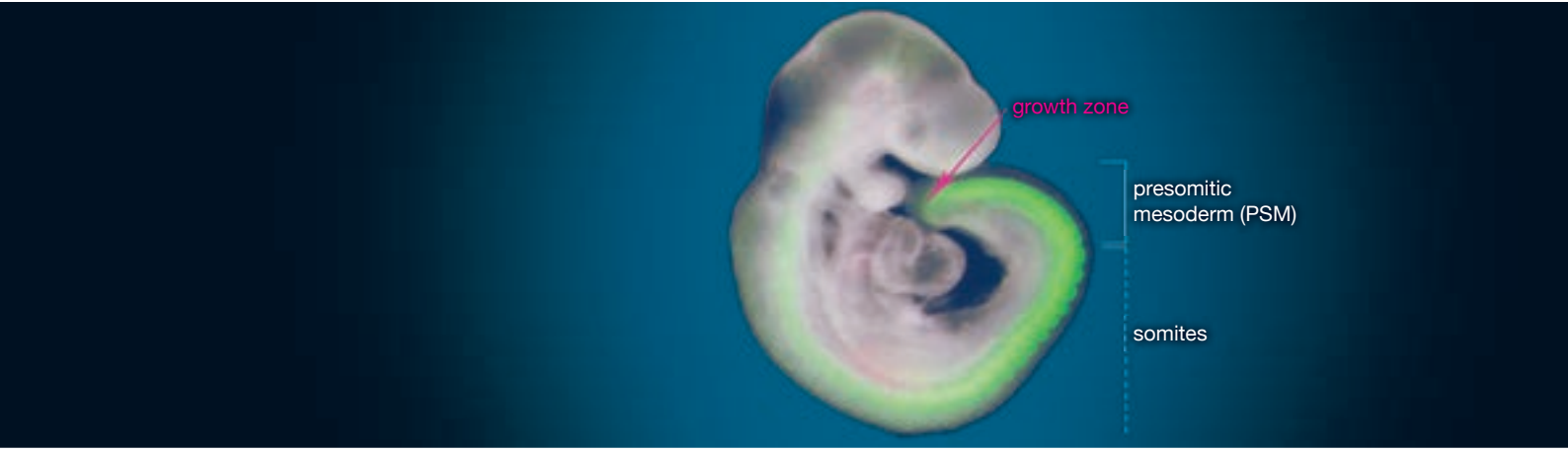


Figure 1: Mouse embryo at day 9.5 of embryonic development.
PSM and somites are stained green (fluorescent marking with GFP) (Source: G. Przemeck).

Gradients in the PSM

In the following years, more and more oscillating genes in the PSM were found. But, it is not clear whether all are equally important or whether there is a main oscillator that controls all the others. Moreover, two candidates for the signal emitted from the tail end were found, the Fgf and Wnt signalling pathways. In both cases, cells release molecules that attach to surface receptors and can trigger further processes in receiving cells. However, the mechanism whereby a signal decreasing from back to front arises is particular; also here molecular degradation plays a role. The PSM does not grow in its entirety, but only in a posterior growth zone. Only there, the *Fgf* and *Wnt* genes are switched on and produce messenger RNA. As these RNAs have a long half-life of more than two hours, their slow degradation leads to a posterior to anterior decreasing gradient in signal intensity in the PSM outside the growth zone.

Interplay of oscillator and gradient – LED ticker in the presomitic mesoderm

A further peculiarity of gene expression in the PSM is the fact that the oscillatory genes do not oscillate synchronously throughout the PSM. Rather, a kind of contracting wave runs from back to front and comes to a halt behind the future somite. In an earlier study we showed that this wave comes about like one seen in a LED ticker consisting of chains of individual LEDs (Tiedemann *et al.*, 2007). Each cell (corresponding to an LED in the ticker) oscillates, but the front ones oscillate more slowly than those at the back, which gives the impression of a moving wave. Our theory is that one of the gradients in the PSM is responsible for influencing the cell degradation processes. In turn, the degradation rates of protein and messenger RNAs play an important role in determining the period of cellular oscillators and in the genetic negative feedback processes described above, respectively.

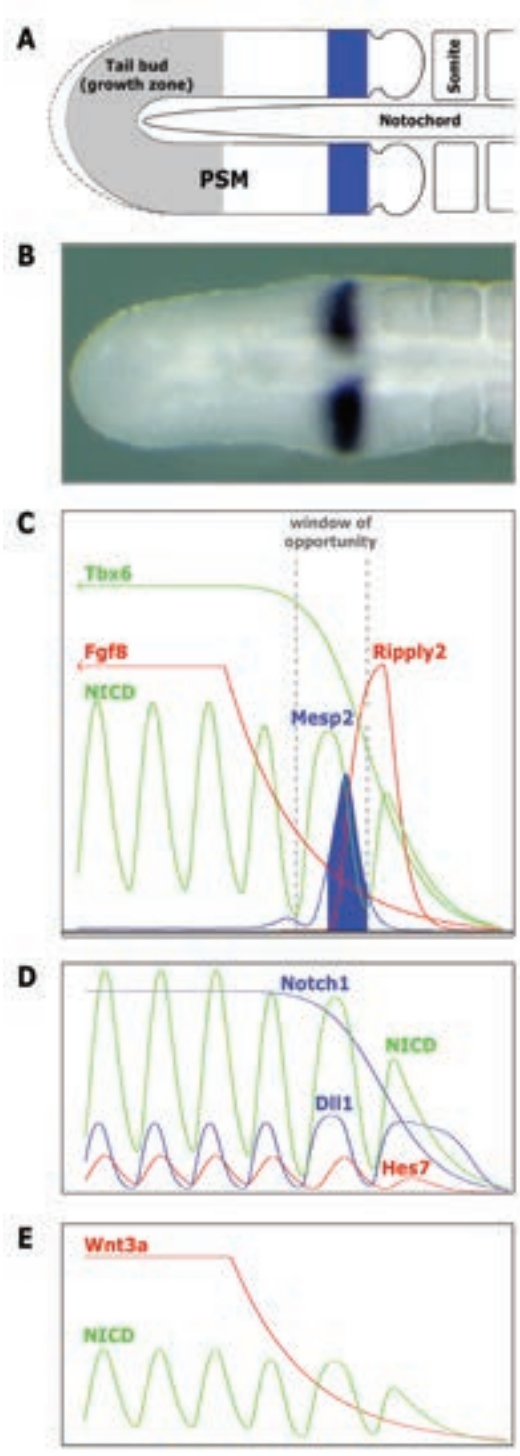
Synchronisation of the molecular clock

That all sounds relatively simple, but is in fact rather more complicated. The oscillating genes in each cell must at least be synchronised at the same length of the PSM, as otherwise all cells would "flash" wildly in a chaotic way and no pattern and no defined wave front would be recognisable. It was ascertained in experiments that the Delta/Notch signalling pathway is responsible for this. Embedded in the cell membrane are ligands and receptors. If a Delta ligand (such as DLL1) of a cell binds to the Notch receptor of an adjacent cell, a cleavage site for an enzyme becomes accessible in the Notch molecule. This leads to the release of the intracellular domain of the Notch molecule (NICD), which migrates to the cell nucleus and binds to the promoters of the *Hes* genes and enables them to be read. Since a molecular signal is transmitted when ligands of a cell bind to receptors at another cell, the possibility arises of coupling the oscillators of various cells of the PSM and thereby synchronising them. This leads simultaneously in the PSM to a wave of "activated Notch" (NICD), which also moves from back to front, contracts in the direction of the longitudinal axis of the PSM and comes to a halt in front of the next somite boundary to be formed.

The effector

The actual effector of boundary formation is the *Mesp2* gene, which ensures that the cells in the PSM move apart at its anterior expression boundary to form a gap that separates the new somites from the rest of the PSM. Analysis of the *Mesp2* promoter revealed that the gene is activated by the joint action of NICD and TBX6 and can be inhibited by the action of FGF8. Furthermore, the *Tbx6* gene is induced throughout the whole PSM by means of the Wnt signalling pathway. Since the TBX6 protein has an activating effect on the whole PSM and FGF8 an inhibiting effect only in its posterior part, the result

Figure 2: The connection between temporally and spatially limited gene expression in somitogenesis



is a limited area in the anterior part of the PSM that is migrating toward the rear where *Mesp2* can be activated if NICD is also present (Oginuma *et al.*, 2010). Since the NICD wave periodically encounters the anterior portion of the PSM where TBX6 enables activation in the abovementioned manner, the result is a sharply restricted periodic *Mesp2* expression that, while migrating towards the rear, appears regularly like clockwork and induces new somite boundaries (Fig. 2).

Simple computer models map only part of observations

Simple computer models have simulated both *Mesp2* induction by means of dynamic NICD expression and the negative feedback of the *Hes* genes on themselves. However, some parts always had to be inserted "manually," as for example periodic functions that stand for NICD expression, or experimentally determined oscillatory behaviour for *Hes7*. So, these models did not explain exactly how the NICD wave comes about. Moreover, simple models are also unsatisfactory in that they describe no complete causal chain and did not clarify whether all sub-processes really interact as thought.

Cell and gene-based computer simulations of somitogenesis help to identify the central oscillator

We have, therefore, developed a cell-based computer model in which the essential molecular processes in the PSM are described by means of differential equations (Tiedemann *et al.*, 2012). These are simultaneously solved numerically in many hundreds to thousands of 'virtual cells'. In addition, the 'cells in the computer' can multiply in the growth zone of the PSM, communicate with each other via Delta ligands and Notch receptors, and show the concentration of any selected gene product by means of colour intensity (virtual *in situ* staining).

Taking experimental findings from mouse embryos as a starting point, we postulate in our model that *Hes7* is the central oscillator in the PSM, with a negative feedback of the HES7 protein not only to its own expression but also to the *Delta* promoter. We also assume that the Wnt gradient influences NICD degradation. These assumptions produce in our virtual PSM the characteristic expression patterns of NICD, *Hes7, Dll1, Mesp2* and other genes (Fig. 3). Furthermore, this enables a

The diagram in **A** shows the PSM with preformed somites (right), the growth zone (left) and a forming gap where the anterior expression boundary of *Mesp2* induces the future somite boundary. **B** shows *Mesp2* expression in the PSM of a mouse embryo. **C**: *Mesp2* is activated by dynamic NICD expression and TBX6 and inhibited by FGF8 and RIPPLY2. **D**: NICD oscillates since it emerges from the reaction between NOTCH1 and DLL1. The expression of *Dll1* is dynamic because in our model it is influenced by the negative feedback oscillator *Hes7*. **E**: The expression of NICD appears wave-shaped because oscillations in the cells are slowed down by the WNT3A gradient. (A, C, D, E taken from Tiedemann *et al.* (2012), PLoS Comput. Biol. *8*, e10002586, doi:10.371/journal.pcbi.1002586.g001)

better understanding of somitogenesis malfunctions that can lead to malformations of the spinal column in humans if the hands of the molecular clock are 'turned' by, for example, mutations in the genes *Hes7*, *Mesp2* or *Lfng*. *Lfng* also plays a role in our model, but has not been discussed here due to space restrictions.

Outlook: Computer models contribute to a better understanding of embryonic development

Of course, our model is only a first step towards a complete understanding of segmentation in vertebrates. Many processes have yet to be explained in detail and quantitative information on the decay and formation rates of many gene products is only partially known. Nevertheless, computer models like ours will become better and better with time and provide the opportunity to shed light on other areas of developmental biology as well. Hypotheses derived from experiments can then be tested, their consequences be calculated and made quantitatively representable.

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The research project in brief:

The project is being carried out by the *Functional Genetics* research group at the Institute of Experimental Genetics, Helmholtz Zentrum München (German Research Center for Environmental Health) and was funded in part by the Helmholtz Alliance on Systems Biology (CoReNe network).

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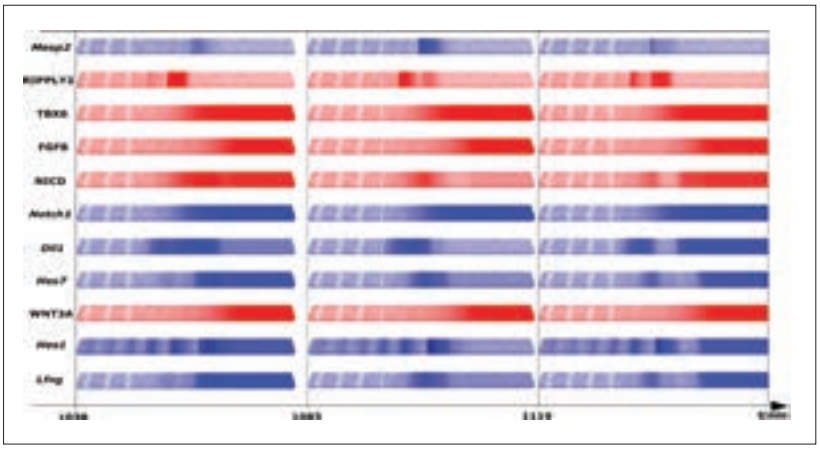
Figure 3: Virtual expression patterns at three different time points in one cycle of the somitogenesis clock



The illustration shows one half of the PSM growing towards the right. Concentrations of messenger RNA are shown in blue and those of proteins in red. (Figure taken from Tiedemann *et al.* (2012), PLoS Comput. Biol. *8*, e10002586, doi:10.371/journal.pcbi.1002586.g003)

systems biology in luxembourg – the luxembourg centre for systems biomedicine (LCSB)

Luxembourg's biomedicine initiative gives rise to a new research institute and a centre of excellence in biomedicine

by Philippe Lamesch and Hannes Schlender

It all started around three years ago, when Luxembourg was given the go-ahead to open a new research institute: the Luxembourg Centre for Systems Biomedicine (LCSB) headed by Prof. Dr. Rudi Balling. The LCSB aims to significantly advance personalised medicine, focussing wholly on Parkinson's disease (PD). The institute is embedded in the Biomedicine Initiative of the Grand Duchy of Luxembourg. Biologists and physicians collaborate with computer scientists, mathematicians and engineers in investigating complex biological systems. The central questions are: How do diseases arise? How do they announce their arrival? How can they be treated in a patient-specific way? These are core questions of systems biology. Equipped with powerful computers and state-of-the-art analysis technology, the Luxembourg scientists and their international partners aim to examine and understand the interactions between genes, proteins and metabolites, thereby making a major contribution to the personalised medicine of the future.

Initially, in September 2009, the LCSB consisted of a sole individual – Professor Dr. Rudi Balling. A distinguished geneticist, Professor Balling moved from his post as Director of the Helmholtz Centre for Infection Research in Braunschweig to an almost empty office at the University of Luxembourg, where he started building up the LCSB from scratch. This was a fundamental step for a renowned scientist and research manager such as Balling. But besides being unable to resist a 'blank canvas', he was also aware of the scale and potential of the Luxembourg research initiative. He was so impressed by the Luxembourg ministries' determination to invest in the fields of systems biology and biomedicine, that he left his established position in German science and moved to Luxembourg.

Since his arrival, the young research centre, which is also part of the University of Luxembourg, has grown continuously. Now, more than 100 research scientists and other staff work at the LCSB and the number continues to grow. With this growth, Balling is pursuing a clear objective. The aim is that in five years' time, the research location of Luxembourg will be a globally recognised entity in the field of biomedicine and its research findings will be of interest to industry and lead to outlicensing and product developments. For Luxembourg, this would mean a return on investment and a diversification of its economy, which until now has specialised heavily in services, primarily in the banking sector. In order to anchor the LCSB as quickly as possible in international science, Luxembourg is investing heavily in knowledge transfer. As a result, it is acquiring biomedical expertise that was previously lacking. A strategic partnership with the world-leading Institute for Systems Biology (ISB) in Seattle has ensured that LCSB's science project got off to a flying start. LCSB scientists are conducting research not only in Luxembourg, but also at the ISB in Seattle, where they have the opportunity to learn about state-of-the-art technologies and ensure a lively exchange of research ideas and concepts.

What lies behind the Luxembourg biomedicine initiative? The country is investing €140 million in the programme, a considerable sum for the small Grand Duchy. Until now, Luxembourg has been known as a location for banking operations and a centre of various EU activities. Research and science have played a minor role. However, that began to change a few years ago when the government paved the way for the development of science, with the University of Luxembourg as the driving force. A comprehensive analysis of the most relevant research fields commissioned by the Department of Trade and Industry resulted in the Luxembourg Health Science Plan, in which the Luxembourg Centre for Systems Biomedicine plays a leading role. This is because biomedicine was identified as the sphere of activity with the greatest future potential. The aim for the LCSB's research is to be the seed for future economical activities in the fields of life sciences and high technology.

Dr. Paul Antony, member of the experimental neurobiology research group at the LCSB, with the Opera optical path microscope (Photo: Dirk Hans/LCSB).

The LCSB in Luxembourg is by no means alone in its ambitious mission. Its strategic partnership with the ISB in Seattle and cooperation arrangements with the Massachusetts Institute of Technology (MIT), Cambridge University and the Systems Biology Institute in Tokyo are part of a vibrant network originating in Luxembourg. This is because the government is not only investing in the LCSB. Some of the money has been used to develop the Integrated Biobank of Luxembourg (IBBL), which collaborates with hospitals at home and abroad to gather tissue and blood samples from patients that are invaluable to scientists. The Centre de Recherche Public Santé and hospitals such as the Centre Hospitalier de Luxembourg, whose expertise has been acquired over many years, contribute clinical know-how to this consortium. With the foundation of the Personalized Medicine Consortium, the network was also given a name.

Science at the LCSB

Since the challenges of competitive biomedical science are best met by linking various disciplines, the LCSB promotes the integration of different technologies, models and skill-sets. These skills range from mathematical theory to bioinformatics and molecular biology. One of the LCSB's primary scientific goals is to use mathematical and computer science methods to develop expertise in modelling disease processes. This is a critical and essential component of future scientific work at the LCSB.

Furthermore, Balling is very aware of the important role that physicians play in biomedical research . 'The integration of medicine into biomedical science is one of our main priorities', states Balling. While the observations of medical doctors in providing daily clinical care can steer scientists in important new directions, the interaction between scientists and physicians also supports the speedy translation of scientific discoveries into clinical applications.

Understanding cellular malfunctions in Parkinson's disease

The main focus of the LCSB's scientific work is on understanding the molecular and cellular foundations of neurodegenerative diseases, focussing especially on Parkinson's disease (PD). Although approximately seven to ten million people worldwide suffer from the disease, research in this field is still in its infancy. The precise multifactorial causes that trigger PD are not sufficiently understood. As a result, there is no cure for this neurodegenerative disease. The main criteria for defining the main focus of research at the LCSB were the medical relevance of a cure for neurodegenerative

In summer 2011 the LCSB moved into its new laboratory building, the House of Biomedicine in Esch-sur-Alzette. The interdisciplinary centre is a pioneer for the University of Luxembourg because in a few years' time the Belval campus will be the university's main site. It is being built amidst old industrial plants that are testimony to the history of steel industry in Luxembourg (Photo: LCSB).

diseases, and the need for an interdisciplinary approach to study diseases, which are triggered by multiple factors. In their research on PD, the LCSB scientists are currently concentrating on characteristics that hold the greatest promise of a breakthrough in the understanding of the disease. New scientific findings suggest that mitochondrial malfunctions play an important role in the pathogenesis of neurodegenerative diseases such as PD and Alzheimer's. Some of the currently known familial Parkinson's genes are responsible for mitochondrial tasks such as fusion/splitting, energy generation and response to cellular stress. The LCSB has adopted a number of different approaches in order to explain this mitochondrial dysfunction in PD. Balling's research group is currently conducting a project in which researchers are analysing gene expression networks based on mitochondrial dysfunction. Their goal is to separate these genes into functional groups that represent different physiological processes connected to mitochondrial dysfunction.

Parkinson's and Alzheimer's are typically accompanied by inflammation. Various investigations suggest that inflammation mediators from non-neuronal cells, including microglia and astrocytes, modulate the advance of these diseases. However, whether the observed inflammation is at the root of the disease or is just a secondary effect of primary neurodegeneration remains to be clarified. To gain a better understanding of the link between neuroinflammation and neurodegeneration, researchers at the LCSB study the cellular components of degenerating neurons and measure the metabolic changes during neuroinflammation. Identifying new metabolic pathways that play a role in neurodegenerative diseases is one of Dr. Karsten Hiller's main goals. His laboratory has developed a mass spectrometry metabolomics platform that can be used to measure metabolic dynamics in cells. Scientists in Hiller's laboratory have used this method to identify a previously unknown metabolic pathway that plays a role in mammalian immune cells during inflammation. A protein with a previously unknown biological role in this process catalyses the production of the antimicrobial metabolite itaconic acid. Scientists at the LCSB are currently further investigating the role of this metabolic pathway in neurodegenerative diseases.

Discovering common disease mechanisms by means of bioinformatics

There is increasing evidence that certain molecular processes may be responsible both for different neurodegenerative diseases and for other diseases such as diabetes. Phenomena such as mitochondrial inflammation or malfunctions have also been observed in other chronic diseases. This would seem to indicate that neurodegenerative diseases may be part of a continuum of phenotypical chronic diseases. Scientists in Dr. Reinhard Schneider's group are currently engaged in a textmining project in which hundreds of complete biomedical publications are being analysed. With the aid of these bio-computational analyses they aim to identify molecular and cellular networks that may be shared by several diseases.

Do microbial communities have an effect on Parkinson's disease?

Scientists agree that the enteric nervous system, also known as the intestinal nervous system, plays an important part in the pathogenesis of neurodegenerative diseases as a 'second brain'. Particular importance is attributed to the role of microbial communities in the intestine that exercise a powerful influence on the immune response of cells in the intestinal mucosa. At the LCSB, Dr. Paul Wilmes has launched a research project to study the cross-talk mechanism between the intestinal epithemial cells and the intestinal microbiome. For this purpose, he developed an *in vitro* microfluidic device (HuMiX) to co-cultivate differentiated human epithelial cell lines and samples of human microbial communities for a longer period. By means of this device Dr. Wilmes and his research group aim to reconstruct models of the human proximal colon, the entire human gastrointestinal tract and human gastrointestinal tissue.

Prof. Dr. Rudi Balling, Director of the Luxembourg Centre for Systems Biomedicine (LCSB). The renowned geneticist came to Luxembourg in 2009 to establish the LCSB. He and his team focus their research on Parkinson's disease with the vision of taking new approaches to treatment – from basic research to clinical application (Photo: Dirk Hans/LCSB).

The PD Map: a knowledge repository on Parkinson's disease

In addition to the projects under way in the individual laboratories, several flagship projects are in progress at the LCSB in which scientists from different disciplines, such as molecular biology and bioinformatics, collaborate. One such project involves the development of a PD Map, a knowledgebase of Parkinson's disease that provides an overview of the relationships between the different pathological factors of the disease and presents them as connected modules. As the first biological map to contain all known molecular components and metabolic pathways that play a part in the disease, the PD Map thus serves as a navigation and exploration tool. It enables users to conduct detailed research into different areas of the disease and to draw up new hypotheses. 'This map can serve scientists as a starting point from which to undertake in-depth biocomputational analyses and develop joint collaborations,' said Dr. Marek Ostaszewski, who is in charge of the project. It is a kind of navigation system that provides scientists at the LCSB and around the world with orientation in their endeavours to understand the causes of Parkinson's disease and to develop new cure concepts for the benefit of the patient.

The research project in brief:

Founded in Luxembourg in 2009, the LCSB is an interdisciplinary research centre where biologists and medical specialists collaborate closely with computer scientists, mathematicians and engineers to conduct research into complex biological systems. The LCSB's main research focus is on Parkinson's disease.

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using systems biology to obtain an understanding of ageing-associated diseases

Presenting the Cologne-based Sybacol research core

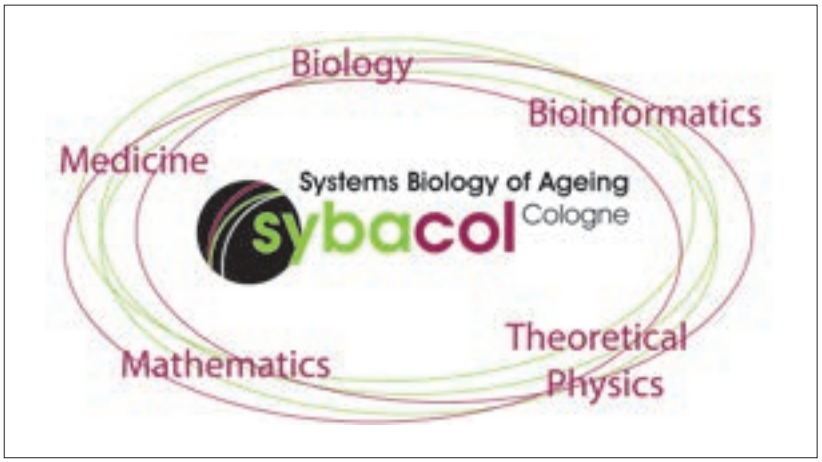
by Martin Höhne

The steady increase in life expectancy since the mid-19th century is without a doubt a triumph of healthcare in general and medical care in particular. It also means, however, that an increasing number of people are growing old enough to suffer from ageing-associated diseases. A large number of age-related illnesses such as diabetes, atherosclerosis, heart failure, cancer, kidney diseases and neurodegenerative diseases such as Alzheimer's, dementia or Parkinson's confront doctors already. With the rising proportion of old people in the population there can be no doubt that more and more people will suffer from these ageing-associated diseases. It is expected that the dramatic increase in ageing-associated diseases will pose a huge economic problem for our healthcare system. It is thus essential to gain a better understanding of the ageing process at the cellular and molecular level in order to develop new strategies for the prevention and treatment of ageing-associated diseases. The BMBF appreciated the importance of this issue at an early stage and that led to the development of the GerontoSys funding programme to promote the "use of systems biology for health in old age" (Boukamp *et al.*, 2011).

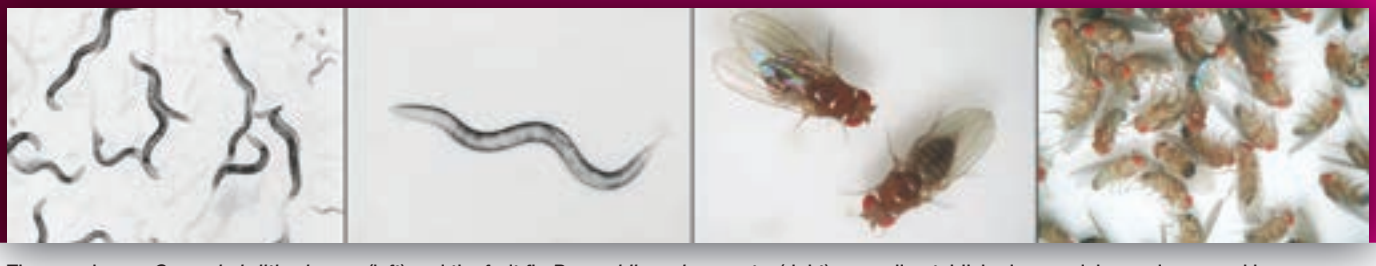
Sybacol: Systems Biology of Ageing Cologne

In the successful Excellence Cluster in Cellular Stress Responses in Aging-Associated Diseases (CECAD), the Max Planck Institute for Biology of Ageing, the Max Planck Institute for Neurological Research with its new focus on metabolic diseases and the University Hospital with its focus on cancer, kidney and metabolism research, Cologne has a large number of institutions that deal with research into ageing-associated diseases. The city has developed accordingly into a leading international centre of ageing research. Furthermore, the proximity of the University's Departments of Theoretical Physics with their longstanding tradition and their own collaborative research centres dealing with biological issues and the internationally renowned Cologne Center for Genomics makes Cologne an ideal location for research into systems biology aspects of the biology of ageing. Convinced that re-

Figure 1: Sybacol is a multidisciplinary consortium



At the Sybacol research core, experimental and theoretical disciplines are represented that collaborate on systems biology aspects of the biology of ageing (Chart: MedizinFotoKöln, University Hospital of Cologne).



The roundworm *Caenorhabditis elegans* (left) and the fruit fly *Drosophila melanogaster* (right) are well-established as model organisms used in gerontology (Photo: *C.elegans* – F. Fabretti; *D. melanogaster* – M. Höhne).

search into the complex connections of the ageing process and of ageing-associated diseases cannot be conducted solely using conventional methods but require the interactive collaboration of theoretical scientists with doctors, biologists and geneticists, Cologne-based scientists, led by Professor Thomas Benzing, Director of the Department II of Internal Medicine at the University Hospital of Cologne, joined forces to set up the Sybacol network (Systems Biology of Ageing Cologne), which is one of the two GerontoSys2 research cores. In this research core, scientists from a wide range of disciplines on the Cologne campus – medicine, biology, physics, mathematics and bioinformatics – have jointly taken up this new challenge (Fig. 1).

Unresolved issues

The central aim of the Cologne researchers is to gain a dynamic picture of ageing processes and ageing-associated diseases that is based on interaction of signalling pathways. This aim goes far beyond the conventional functional characterisation of individual genes and can only be achieved by means of a systems biology approach. Major progress would have already been made if it had been possible to answer the following questions: Which gene expression networks are in charge of determining lifespan? How do 'longevity genes' influence the stochastic accumulation of DNA damage and its repair? What influence do other pathways have on longevity (pathways) and which physiological consequences arise from interactions between them? And, finally, how do changes in lifestyle and the environment affect these pathways?

Answers from Cologne

It is quite clear that these questions go beyond any answers that the Cologne research core or the GerontoSys initiative will be able to provide in the foreseeable future. This is why two core topics of central significance were singled out for the Sybacol initiative. Research into them is undertaken in two project areas: Area A – *Systems Biology of Longevity Pathways* und Area B – *Quantitative Modelling of Insulin Signalling in Longevity and Disease*.

Project Area A: Systems Biology of Longevity Pathways

This project area is coordinated by Adam Antebi, Director of the Max Planck Institute for Biology of Ageing, and Björn Schumacher, a professor at CECAD, and deals with the systems biology of signalling pathways that influence lifespan. But the "principal player" in the project area is *Caenorhabditis elegans*, a roundworm about 1mm long.

Nematode research into ageing

Research into the model organism *C. elegans* has led to an enormous increase in knowledge in biology in recent decades, as is evidenced by the number of Nobel Prizes awarded for knowledge gained by work on this organism: Sydney Brenner, H. Robert Horvitz, John E. Sulston (2002); Andrew Z. Fire, Craig C. Mello (2006) and Martin Chalfie (2008). For research into ageing, the roundworm, or nematode, *C. elegans* with its relatively brief lifespan of two to three weeks is a stroke of luck. Research into the regulation of this nematode's lifespan has revealed that different signalling pathways affect it. Increased or reduced activity by these pathways, such as the insulin pathway, or the HIF (Hypoxia Inducible Factor) pathway, can result in a drastic increase or decrease in lifespan (Fig. 2). Interestingly, these pathways are strongly conserved in evolutionary terms and are also to be found in yeasts, fruit flies and humans. We know that these different pathways are non-redundantly incorporated into an overriding signalling network that plays a role in vertebrates, not only in regulating lifespan, but also in the occurrence of ageing-associated diseases. However, many issues that are being pursued in this project area are still unresolved.

Do common denominators exist that prolong lifespan under different paradigms? Precisely which modulations of the characterised signalling pathways are required to prolong lifespan? What is the exact cellular or biochemical correlation between these modulations and exactly when is the point reached at which the disrupted onward signal transmission

has a negative effect and cellular stress gets out of hand? In which tissues do these signalling pathways need to be changed to have an effect on the overall organism? Ageing is also associated with an increase in DNA damage and an accumulation of toxic aggregates, and that must not, of course, be neglected; it is, indeed, incorporated in the models.

Research is also undertaken into the transcription activity, or the extent to which genes are active, across different age levels and different worm strains. For this purpose so-called expression profiles of the protein-coding messenger RNAs (mRNAs) as well as microRNAs, which act in a regulatory capacity, are drawn up and compared. In this way scientists hope to identify signature models and signalling pathways that characterise ageing. These modules can then be modelled *in silico*. The challenge is to develop quantitative, dynamic models of the interacting signalling pathways. These models are required to predict the crosstalk between the different signalling networks and to identify previously unknown functional modules and nodes in these networks.

Project Area B: Quantitative Modelling of Insulin Signalling in Longevity and Disease

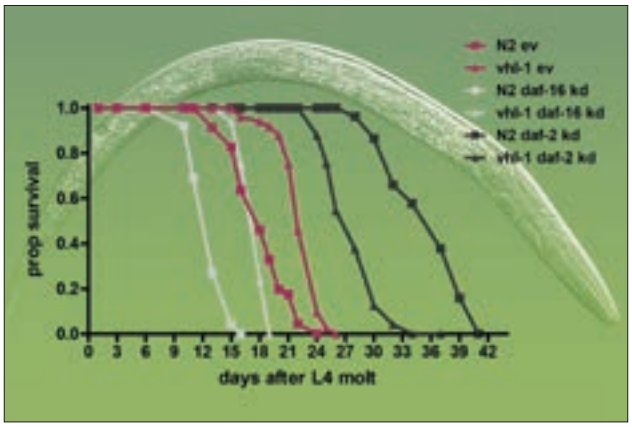
We are all familiar with insulin as the hormone of which the body produces too little in cases of Diabetes mellitus. However, the fact that insulin is one of the most interesting hormones for research into ageing is likely to be something that fewer are aware of. In Project Area B, coordinated by Linda Partridge, Director of the Max Planck Institute for Biology of Ageing, and Jens Brüning, Director at the University Hospital of Cologne and at the Max Planck Institute for Neurological Research, the Sybacol consortium scientists are investigating the known, but as yet not understood insulin paradox.

The insulin paradox

As mentioned earlier, total insulin resistance leads to Type 2 Diabetes mellitus. In contrast, a partial insulin resistance has a positive effect in widely differing model organisms such as the roundworm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* (Fig. 3) or in mice, namely that their lifespan is increased and ageing-associated diseases occur less frequently! How is this paradox to be explained? At least two mechanistic models might help here. The first model assumes that a partial insulin resistance leads to a correspondingly partial impairment or weakening of all insulin-stimulated signalling pathways and thereby to the beneficial effect of insulin resistance on lifespan, whereas a marked impairment of all insulin-stimulated signalling pathways leads in the final analysis to a reduced lifespan and is accompanied by the occurrence of Diabetes mellitus. According to an alternative model, different insulin-stimulated signalling paths need different insulin levels for an all-or-nothing response. With a slightly reduced insulin level, therefore, the signalling pathways that have a positive effect on lifespan might be activated, whereas in the event of a greater reduction in insulin signalling, further pathways are affected and lead to a reduction in lifespan as a result of the occurence of Diabetes mellitus.

The underlying molecular basis for this paradox is still totally unclear. Mathematical models need to be developed to track it down and understand it, and this is a main focus of scientific interest in this project area. The experimental basis for these models must be temporally resolved quantitative data. We cannot "simply" work with insulin resistance yes-or-no models; we must be able to define and regulate the degree of insulin resistance. Quantitative experimental

Figure 2: *Caenorhabditis elegans* is an excellent model for research into ageing



Manipulation of individual signalling pathways has a clearly measurable effect on the lifespan of animals. In the example shown here, the lifespan of typical wild type, i.e. non mutated, worms (red curve ■) is compared with that of worms that lack the vhl-1 gene (an HIF signalling pathway regulator) (red curve ▲). The vhl-1-deficient worms live measurably longer. The white and dark grey curves describe the lifespan of typical wild or vhl-1-deficient worms in which, in addition, the insulin signalling pathway was either activated (white) or weakened (dark grey). Experiments of this kind can provide information about the interaction of different signalling pathways (modified according to Müller *et al.*, JASN 2009).

models of this kind that permit a defined reduction in insulin signalling in the living organism have been and continue to be developed for the model organism *Drosophila melanogaster* and for mice. The data will again be gene expression profiles, i.e. information about which genes are activated under which experimental conditions and to which extent. By means of these experimental systems it is hoped that we will be able to distinguish between the above-mentioned alternative models and whether a gradual weakening of insulin signalling in the defined insulin signalling pathways has the effect of a correspondingly gradual weakening of the insulin response or whether there is an all-or-nothing threshold. As these analyses are also undertaken separately by different organs, they will further provide information about which organ system responds to a certain level of insulin signalling and how strongly it does so.

The Sybacol research scientists are convinced that their questions and their approach will contribute at the level of molecular systems biology to an understanding of the dynamic processes of ageing and the occurrence of ageing-associated diseases, thereby enabling a further step toward identifying new biomarkers and, ultimately, to the development of new active agents and drugs to treat ageing-associated diseases. Only a joint approach with bridges between experiment and theory and the establishment of new models that generate testable hypotheses will enable us to understand the complex processes of ageing and the basics of ageing-associated diseases.

The research project in brief:

Systems Biology of Ageing Cologne (Sybacol) is a research core and part of the BMBF-funded GerontoSys2 programme. On one campus, research scientists from the University of Cologne, the University Hospital of Cologne and the Max Planck Institute for Biology of Ageing are working to gain an understanding of the complex fundamental processes that play a role in ageing.

The interdisciplinary research core bundles the competences of biologists, medics, physicists, mathematicians and bioinformatics specialists. With the Max Planck Institute for Biology of Ageing and the Cologne Excellence Cluster in Aging-associated Diseases (CECAD), the Sybacol research core is embedded in a research landscape that has taken up the challenge to gain an understanding of ageing.

Figure 3: Cultures of the fruit fly *Drosophila melangaster* (Photo: M. Wodak, MedizinFotoKöln, University Hospital of Cologne).

Participating partners: Prof. Thomas Benzing, Prof. Adam Antebi, Prof. Michael Lässig, Prof. Linda Partridge, Dr. Bianca Habermann, Dr. Silvia Gruhn, PD Dr. Bernhard Schermer, Prof. Johannes Berg, Prof. Joachim Krug, Dr. Björn Schumacher, Prof. Jens Brüning
Coordinators: Prof. Thomas Benzing (thomas.benzing@uk-koeln.de), Prof. Adam Antebi (antebi@age.mpg.de), Prof. Michael Lässig (mlaessig@uni-koeln.de)
Project office: Dr. Martin Höhne
Further information: www.sybacol.org

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purple bacteria

Interesting model organisms with biotechnological potential

by Steffen Klamt, Oliver Hädicke and Hartmut Grammel

Purple non-sulfur bacteria are able to adapt their metabolism to a wide range of environmental conditions, enabling them, for example, to switch between photosynthetic and respiratory lifestyle. In view of their extraordinary flexibility and the attendant regulatory mechanisms these bacteria are interesting model organisms for systems biology. Given their potential for producing biohydrogen, biopolymers and photoactive pigments, research scientists are hopeful that a better understanding of purple bacteria will also lead to new biotechnological applications. In close cooperation between experimental and theoretical research at the Magdeburg-based Max Planck Institute, an entire series of new biological insights have been gained in a project funded by the Federal Ministry of Education and Research (BMBF).

Purple bacteria as energy metabolism all-rounders

Purple non-sulfur bacteria are widespread in nature and, by virtue of their metabolic activity, one of the most versatile forms of life. In the presence of oxygen, their growth occurs by means of respiratory metabolism with the aid of the electron transport chain (ETC) in a manner comparable to that of human mitochondria. If there is light instead of oxygen in their surroundings, a total reorganisation of the bacterium takes place. By creating extensive intracytoplasmic membranes (ICM) with light-harvesting complexes and reaction centres, the cells switch to a photosynthetic lifestyle. With light as their source of energy, they generate adenosine triphosphate (ATP) by means of cyclic electron transport. The bacterial chlorophyll and carotinoids in photosynthetically active protein complexes leads to the characteristic purple colouring. Restructuring is dependent on external signals such as oxygen, light intensity or choice of substrate and is controlled via different regulatory mechanisms that monitor and respond to the redox state of the ETC and the entire cell. Other important metabolic pathways in addition to the ETC are carbon dioxide fixation via the Calvin cycle or (partial) reductive citric acid cycle, nitrogen fixation via a nitrogenase (releasing hydrogen as a by-product) and fermentation when neither light nor oxygen is available.

The creation of ICMs in response to the environmental factors oxygen and light is one of the best-investigated model systems for membrane differentiation in bacteria (Fig. 1). Many studies of purple bacteria, especially *Rhodospirillum rubrum* and *Rhodobacter sphaeroides*, have in recent decades made substantial contributions to our present understanding of fundamental metabolic processes. However, it is difficult to intuitively arrive at an integrated view of all components and influencing factors involved and many issues still remain unresolved.

A consequent systems biology approach

As part of the FORSYS initiative at the Magdeburg Center for Systems Biology (MaCS), *Rhodospirillum rubrum* has been subject to intensive investigation as a systems biology model organism for redox metabolism and redox regulation in recent years. In addition, the potential of the bacterium for application in bio-industrial processes has been explored (Fig. 2). Hartmut Grammel's experimental research group and Steffen Klamt's mathematical modelling group cooperated under the aegis of the Max Planck Institute for Dynamics of Complex Technical Systems.

The experimental research focussed on *R. rubrum* cultures (mainly in bioreactors) from which enzymatic profiles were created under aerobic, microaerobic and photosynthetic (anaerobic) conditions. The organisation of the central metabolic pathways under the three conditions named above was characterised accordingly. Furthermore, central redox metabolites such as ubiquinone 10, glutathione and NAD(P)H were recorded quantitatively – partly *in vivo* by means of spectroscopic processes (Grammel and Ghosh, 2008).

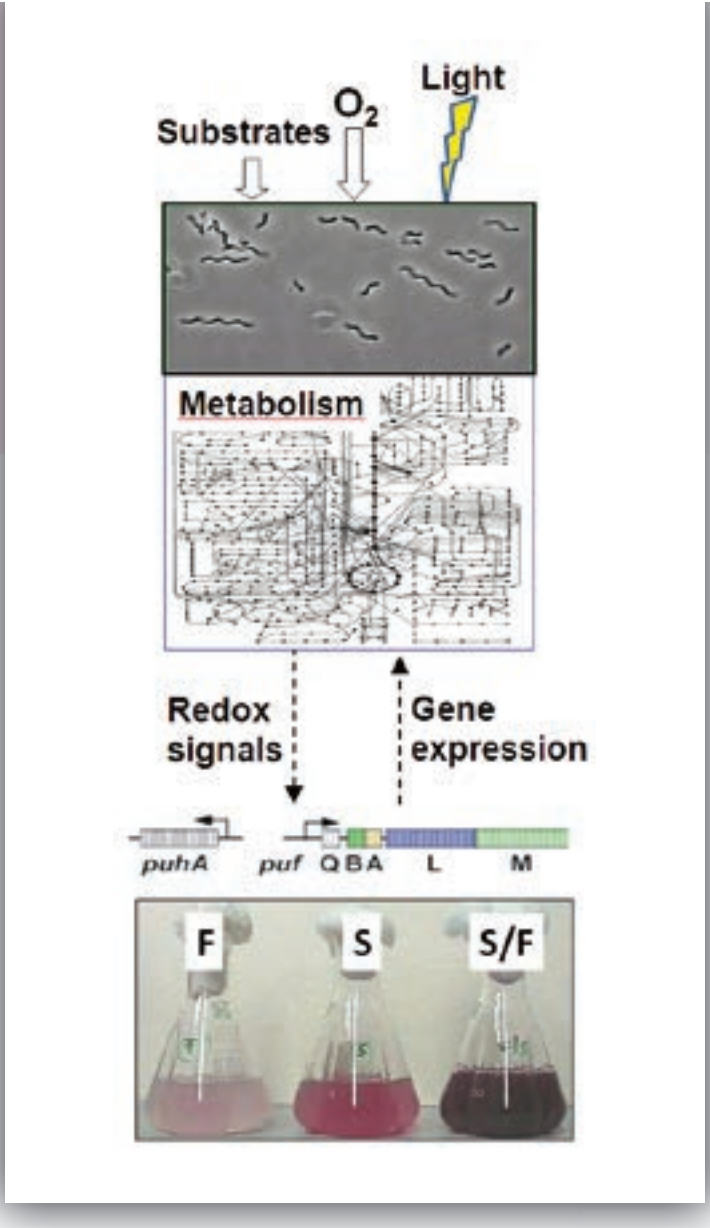

Figure 1: Purple non-sulfur bacteria:
Oxygen, light and substrate are the main environmental factors influencing central cellular redox states and thereby the regulation of the metabolism and the resulting macroscopic phenotype. The photo below shows the phenotype of three different cultures grown microaerobically in the dark on fructose (F), succinate (S) and a mixture of succinate and fructose (S/F) (Chart: Hartmut Grammel).

For *R. rubrum*, a cultivation process was developed that surprisingly permits the production of large amounts of ICM under chemoheterotrophic conditions in the dark and entirely independent of light. In this process, microaerobic oxygen conditions (less than 1% of dissolved oxygen) and a culture medium with two different carbon sources (succinate and fructose) leads to the maximum production of ICM in the dark. Quantities were produced that had previously only been observed in phototrophic cultures (i.e. those grown in the light, see Fig. 1). This phenomenon provides an experimental access to quantitatively investigate how cellular redox conditions influence signal processing and gene regulation.

An electron transport chain model

Based on the experimental investigations, the Klamt group drew up a kinetic model of the ETC (Klamt *et al.*, 2008) in order to gain a better understanding of how the external signals light, oxygen and substrates influence the redox state of central ETC components and thereby activate redox-controlled regulation pathways. Experimental data provided by the Grammel group served to validate the model. A key result was that the model confirmed the longstanding assumption that the redox state of the ubiquinone is a suitable signal for regulating photosynthetic genes. Previously, it was particularly unclear whether the redox carrier ubiquinone was more reduced or more oxidised under low-light conditions compared with high-light intensities. It would need to be the former if the redox state of ubiquinone was to be a meaningful signal for the regulation of gene expression. In fact, the model exhibited and explained this rather non-intuitive behaviour in a robust manner for a large range of parameters. By means of special measurement methods, the Grammel group eventually also confirmed a stronger reduction of ubiquinone under low-light conditions. The model also reproduced the above-mentioned succinate-fructose effect. The cytoplasmic NADH pool rises (as was measured) under this substrate combination and, as a consequence, more electrons find their way into the ETC, leading to a stronger reduction of ubiquinone 10. The special substrate combination thereby causes an effect in the dark that is, in principle, comparable to a reduction of light intensity under phototrophic conditions. These findings may be of general significance for redox regulation processes in bacteria.

A stoichiometric metabolic model

Along with the processes in the ETC, the regulation and adjustment of material flow in the redox and central metabolism are also of great interest. As a much larger number of metabolites and enzymatic reactions need to be taken into account here, and since not enough kinetic information is available, a stoichiometric metabolic model for purple non-sulfur bacteria was created and implemented in the *CellNetAnalyzer* software (Hädicke *et al.*, 2011; Fig. 3). Although this model is only based on the stoichiometry of the most important 120 or so metabolic reactions in these organisms, it proved possible by means of flux balance analysis to simulate

a series of experimentally observed phenomena and thereby to investigate the role of central metabolic pathways under different environmental conditions. A key result was the model-based explanation of why the CO_2-fixing Calvin cycle is essential for photosynthetic growth on certain substrates such as malate or succinate even though a net release of CO_2 is measured under these conditions. Under photoheterotrophic conditions, the Calvin cycle functions primarily as an essential sink for the NADH that is created in the metabolisation of the substrates. The refixation of a part of the CO_2 that is released in the process is effectively only a favourable side effect. With acetate as a substrate, several species of purple bacteria can grow without the Calvin cycle, while others do not. Here, the model also explained how the availability of alternative pathways in some species allows to compensate for a deficient Calvin cycle under these conditions.

Biotechnological applications and perspectives

The potential of the outstanding metabolic versatility of purple bacteria for applications in biotechnology has long been realised and is reflected in numerous publications and entries in patent databases. Fields of application include the production of biohydrogen, biopolymers, coenzymes (Q10), vitamins and recombinant membrane proteins. Porphyrin derivatives from bacterial chlorophyll can also be used in photodynamic tumour therapy.

Especially intensive research has been undertaken into the biological production of molecular hydrogen (H_2). *R. rubrum* has several enzymatic routes that lead to the release of H_2. In particular, the ability of nitrogenase to reduce both nitrogen and protons contributes to its capacity as an H_2 producer. Here, the metabolic model can also be used to calculate the maximal possible H_2 yield from a given substrate (Hädicke *et al.*, 2011). Interestingly, it turned out that the molecular H_2 yield can in fact be higher than the amount of bound hydrogen in the substrate. By means of hydrolysing enzyme reactions, for example, 12 moles of H_2 can be generated from one mole of fructose – six moles directly from hydrogen bound in fructose and a further six moles from water.

A major obstacle to the industrial biotechnology use of purple bacteria has hitherto been the technical difficulty of providing sufficient light for large volumes and high densities of photosynthetic cultures. The dramatic increase in quantities of expressed membranes under microaerobic conditions as described above and the use of succinate-fructose medium in the dark opens up a new prospect of manufacturing large quantities of photosynthetic products in conventional stirred bioreactors without complicated illumination technology. Based on a kinetic process model, the Grammel group was able to set up a fed-batch process that delivers cell densities of around 60 g of dry mass concentrations per litre with *R. rubrum* (Zeiger and Grammel, 2010). This level has so far not

Figure 2: Systems biology approach to the characterisation of redox phenomena in purple non-sulfur bacteria and their biotechnology applications

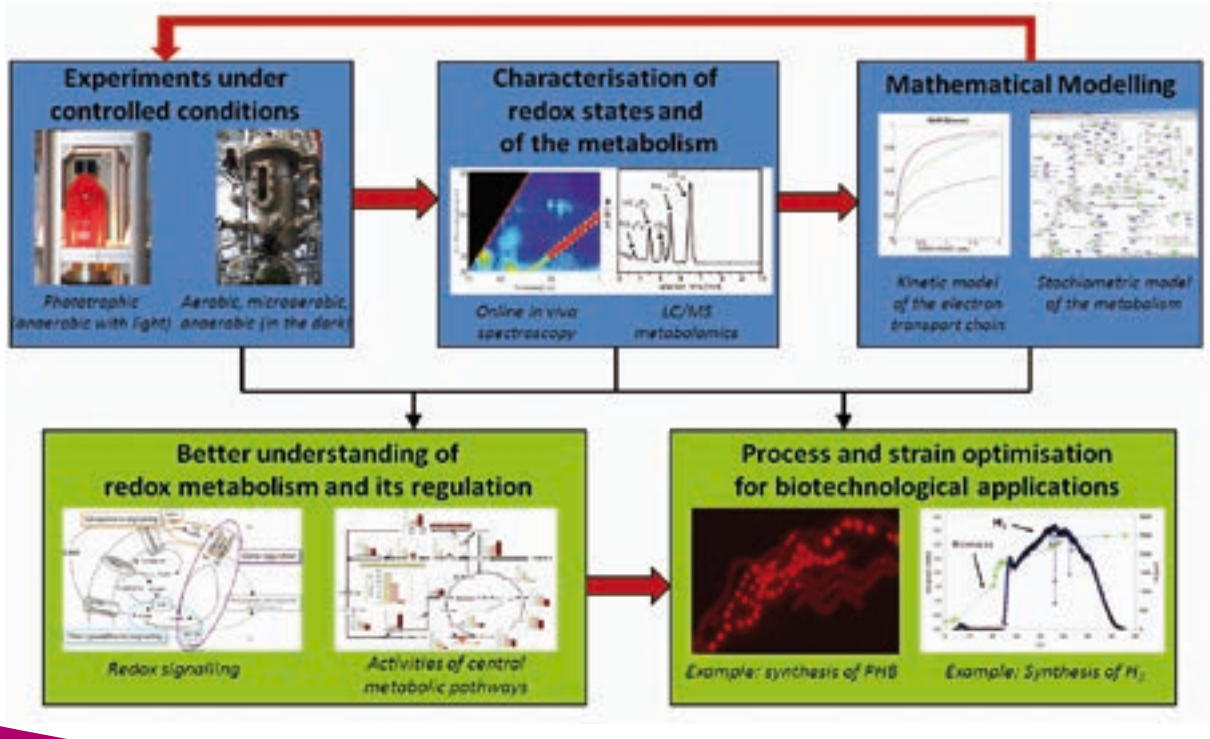

Chart: Steffen Klamt

been achieved with any other photosynthetic microorganism and enters the range of non-photosynthetic organisms commonly employed in bioindustrial processes.

In future, the metabolic model is also to be used for metabolic engineering, i.e., the targeted genetic optimisation of bacteria to boost production of required chemicals. A stoichiometric model supports the search for suitable intervention strategies such as knockouts or overexpression of certain genes. To this end, a new mathematical approach recently proposed by the Klamt group (Hädicke and Klamt, 2011) will be used. This method allows to calculate all relevant knockout strategies for optimizing the yield of a given target product.

The results and preliminary work described form the basis for a new project launched as part of the BMBF initiative entitled "Biotechnology 2020+ – Basic Technologies for a Next Generation of Biotechnology Processes". The long-term vision consists of the cell-free use of CO_2-fixing enzyme systems in purple bacteria to utilise CO_2 as a raw material for electrochemical synthesis of organic recyclables.

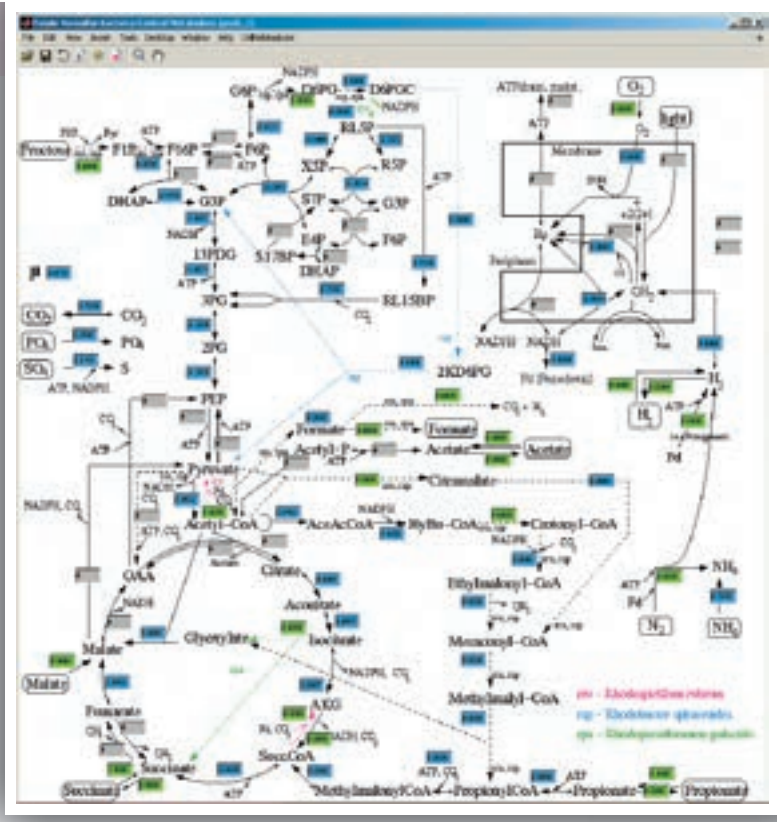

Figure 3: The stoichiometric metabolic model for purple non-sulfur bacteria (*CellNetAnalyzer* screenshot). The scenario shows calculable flows (blue text boxes) of photoheterotrophic growth on succinate (green text boxes indicate predefined (known) flows) (Chart: Oliver Hädicke).

The research project in brief:

Project name: Redox Phenomena in Photosynthetic Bacteria – Systems Biology Research and Biotechnology Applications. The project was initiated as part of the five-year BMBF funding initiative FORSYS – Research Units for Systems Biology at the Magdeburg Centre for Systems Biology (MaCS). Participants were Hartmut Grammel's experimental junior research group "Redox Phenomena in Photosynthetic Bacteria" and Steffen Klamt's group "Analysis and Redesign of Biological Networks" at the Magdeburg Max Planck Institute for Dynamics of Complex Technical Systems. A production system for manufacturing of carotinoids with *R. rubrum* is also being developed within the context of a FORSYS partner project in cooperations with the University of Stuttgart. Further cooperations were established with the "Dynamic Systems – Biosystems Engineering" research centre in Magdeburg.

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News from the BMBF

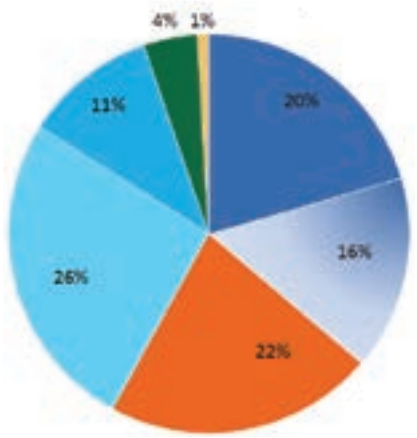
Photo: © Oliver Boehmer – Fotolia.com

The 2013 federal budget

In approving the 2013 federal budget, the federal government has reaffirmed the fundamental importance of education and research. It is one of society's foremost tasks to secure young people's opportunities for the future. We are safeguarding Germany's future by creating good conditions for education, vocational training and study.

Compared with 2012, the 2013 federal budget is up by 6.2% to €13.7 billion with a focus on academic education and research. Under the 2020 Higher Education Pact, the *Länder* will receive about €1.85 billion in 2013 for additional university places. The Quality Pact for Teaching will provide a further €200 million for improving study conditions and the quality of teaching. The Excellence Initiative and programme funding are providing further funds of around €680 million for promoting university research.

Institutional and project funding will also be increased further. Project funding under the High-Tech Strategy will rise to €2.3 billion and the 5% annual growth rate under the Pact for Research and Innovation will be maintained. The focus will be on key societal and global challenges such as climate change, demographic trends, the spread of common diseases, securing world food supplies and the finiteness of fossil commodities and energy sources.



BMBF (Departmental Budget (EPL) 30) – EPL 30 areas of activity for 2013 – €13.740 billion

- Knowledge-oriented basic research across different programmes
- Research and development to provide for the future
- Technology and innovation funding
- University construction and special programmes directed mainly towards universities
- Federal Training Assistance Act (BAföG)
- Other, non-R&D-relevant educational expenditure (excluding BAföG)
- Ministry expenses, including pensions

Research Campus

The ten winners of the BMBF's "Research Campus – Public-Private Partnership for Innovation" funding initiative have been named. Federal Minister Annette Schavan, Henning Kagermann, President of acatech, the National Academy of Science and Engineering, and Ernst Rietschel, Emeritus President of the Leibniz Association, jointly announced the jury's decision.

FORSCHUNGS CAMPUS
öffentlich-private Partnerschaft für Innovationen

The cooperation arrangements between universities, research facilities and business enterprises span periods of up to 15 years and cover a wide range of scientific issues: sustainable mobility and production of the future (ARENA 2036), smart home networking (Connected Living), lasers in production and component manufacture (Digital Photonic Production), sustainable energy technology (Power Grids of the Future), connecting smart grids and electromobility (EUREF), swift and efficient proof of infectious agents (Infeco-Gnostics), molecular medical intervention environments (M2OLIE), modelling, simulation and optimisation in logistics and medical technology (MODAL AG), hybrid lightweight construction (Open Hybrid LabFactory) and image-guided minimally invasive medical techniques (STIMULATE).

The ten successful consortiums made the running in competition with 80 rivals. The jury chairmen emphasised the high quality of the applications.

Further information at:
http://www.bmbf.de/press/3350.ph

Reading and Writing – My Key to the World

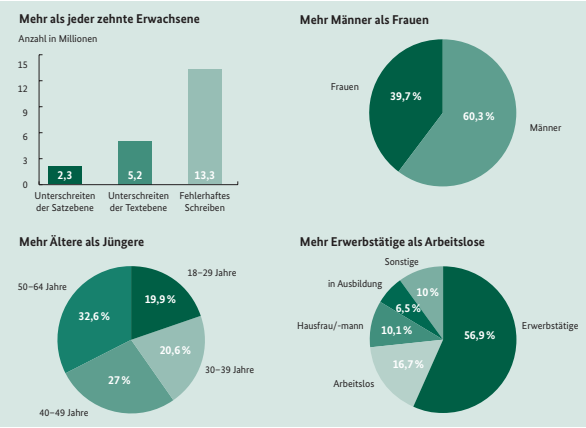
More than one in ten employable Germans have no or only inadequate reading and writing skills, and a further one in four makes mistakes in writing even common words.

With its nationwide awareness-raising campaign "Reading and Writing – My Key to the World", the BMBF is showing that learning to read and write opens up both personal and career prospects and that it is never too late to open up these 'doors to the world' yourself. The campaign is part of the federal government and *Länder* "National Strategy for Literacy and Basic Adult Education in Germany" and is aimed primarily at removing the taboos from the subject of illiteracy and drawing attention to the many support programmes.

A specially developed interactive exhibition has been touring the *Länder* and local communities since October 2012. Working in association with Germany's partners in literacy, the exhibition provides information at regional events about everything that is worth knowing on the subject.

Further information at:
http://www.bmbf.de/press/3346.php
http://www.bmbf.de/de/426.php
http://www.mein-schlüssel-zur-welt.de

7.5 million people in Germany cannot read and write properly.



Source: leo.Level One Study, University of Hamburg
Photo: German Federal Ministry for Education and Research (BMBF)

Education at a Glance 2012

The annual international OECD publication "Education at a Glance" deals with educational policy issues and provides orientation in assessing and developing the educational system. "The latest report testifies to the performance of the German educational system," said Cornelia Quennet-Thielen, State Secretary at the BMBF, speaking at the presentation of the OECD's statistics. "From early childhood to further education, participation in education in Germany is above average. That is an essential prerequisite for our country's economic success." The OECD report demonstrates that individual incomes and social returns are higher and the risk of unemployment lower the higher the level of education.

Germany scores high marks in several categories. In 2010, for example, 89% of three-year-olds attended an elementary education facility (OECD average 66%). With an educational participation of 51% among 15 to 29-year-olds, Germany is also above the OECD average (47%). Not only has the number of new students set a new record (2011: 517,000); since 1995, the proportion of university graduates has more than doubled to 30%.

Further information at:
http://www.bmbf.de/press/3338.php

Demographic change / third Public Dialogue

Few trends will make a more significant mark on Germany in the years ahead than demographic change. An ageing society and population decline cannot be influenced much in the short term, but politics and society can play an active role in shaping the consequences of demographic changes.

The Public Dialogue has an established role as an instrument for societal inclusion. Focussing on the central question of "How is our country to remain rich in ideas and innovative?", members of the public exchange views about the opportunities and challenges of demographic change with experts at conferences and on the internet. The recommendations arising from this Public Dialogue are passed on to decision-makers in politics, business, science and research as orientation points for demographic strategy.

Further information at:
http://www.bmbf.de/press/3348.php
http://www.buergerdialog-bmbf.de/demografischer-wandel/index.php
http://www.bmbf.de/de/20112.php

Super MUC

Germany has consolidated its strong position in the area of supercomputers with the launch of the Super MUC supercomputer at the Leibniz Data Center of the Bavarian Academy of Sciences in Garching near Munich. Twenty of the world's 500 fastest computers are in Germany; two of them even rank among the Top Ten.

The IBM SuperMUC performs three trillion mathematical operations (three petaflops) per second and comes fourth in the global rankings. Garching has been part of the national Gauss Centre for Supercomputing (GCS) together with partner centres in Jülich and Stuttgart since 2007 and is a member of the European supercomputer network "Partnership for Advanced Computing in Europe" (PRACE).

The federal government and the *Land* of Bavaria will share the procurement and operating costs of the supercomputer in the years ahead – about €135 million.

Further information at:
http://www.bmbf.de/press/3316.php
http://www.bmbf.de/de/298.php

Green Economy

One aim of the so-called Green Economy is to maintain or even increase productivity with fewer raw materials, fewer harmful emissions and a lower input of energy. This approach is already a genuine competitive factor as far as Germany is concerned. The country has a 23% share of the world market for eco-friendly energy and energy storage. Over two million people are employed in the environmental industry. Project funding for sustainability research has nearly doubled over the past eight years to its present level of about €430 million.

To further accelerate this trend, Federal Research Minister Annette Schavan and Federal Environment Minister Peter Altmaier invited around 450 experts from science, business, politics, trade associations and society to attend a two-day conference in Berlin in September 2012 entitled "Green Economy – A New Economic Miracle?"

In doing so, the ministers were following up on a result of the United Nations Rio+20 summit, which saw the Green Economy as a central strategic instrument for sustainable development. They are preparing a joint agenda process to examine all areas of economic activity in Germany and in the international context and compare the various perspectives of finance, employment, production and consumption. For this purpose the BMBF is planning to adapt its research funding under the Research for Sustainable Development (FONA) programme accordingly.

Further information at:
http://www.bmbf.de/press/3336.php
http://www.fona.de/green-economy

Contact

For information about these and other interesting aspects of the High Tech Strategy for Germany, visit **www.hightech-strategie.de**

HELMHOLTZ INITIATIVE ON SYNTHETIC BIOLOGY LAUNCHED

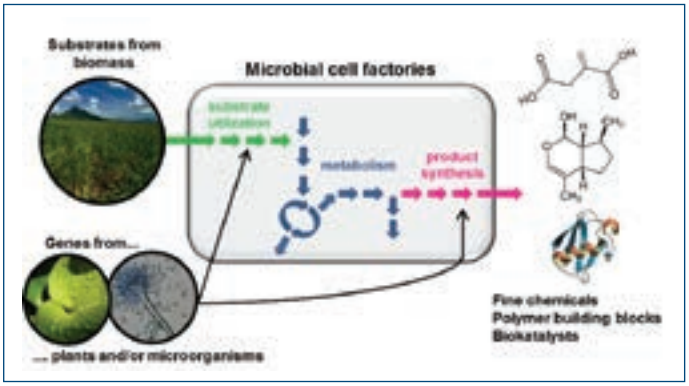
In September 2012 the Helmholtz Association began funding the emerging discipline of synthetic biology. Synthetic biology is regarded as an innovative key technology with a high potential in both fundamental and applied research in health and environment. Prospective applications include faster development of new vaccines, non-fossil fuels and bioremediation, as well as biotechnological applications, such as the cost-effective production of new pharmaceuticals and chemical products. Since synthetic biology, as an emerging life science discipline, has high strategic relevance for the research mission of the Helmholtz Association, the new initiative aims to create sustainable, internationally recognized research structures in Germany.

The new initiative integrates researchers from five Helmholtz centers and two universities, who receive three million Euro from the Helmholtz Initiative and Networking Fund for the next two years; the same amount is contributed by the associated institutions from their own budgets. The aim of the initial phase, which is funded until 2014, is the establishment of a platform for sustainable research structures, which will be integrated as a cross program topic into the program-oriented funding schemes of the Helmholtz research areas Health and Key Technologies.

A combination of life sciences with engineering

Synthetic biology aims towards targeted generation of organisms with new, desired features according to a previously designed blueprint. This requires not only the classical disciplines of theoretical and experimental life sciences, but also engineering as a third discipline. Drew Endy of Stanford University describes synthetic biology as a way of “making biology easy to engineer” (www.openwetware.org/wiki/Endy:Research) - in other words, it is the application of biology for constructive purposes. Thus, simple “biological parts”, such as promoters and protein domains, are assembled to more complex “biological devices”, such as enzymatic cascades and genetic circuits. Those can be integrated into existing organisms, the so-called “chassis”, to fulfill new functions. Recent breakthroughs in the field include the re-programming of the yeast *Saccharomyces cerevisiae* to synthesize a precursor of the antimalarial drug artemisinic acid (Ro *et al.*, 2006) as well as the generation of the first microorganism with a synthetically constructed genome, *Mycoplasma mycoides* JCVI-syn1.0, by the research team of Craig Venter (Gibson *et al.*, 2010).

Microbial cell factories



In biotechnology, bacteria are utilized as environment-friendly microbial cell factories. The application of synthetic biology methods enables the incorporation of new metabolic pathways based on genes from different organisms and thus allows the effective production of valuable metabolites from biomass.

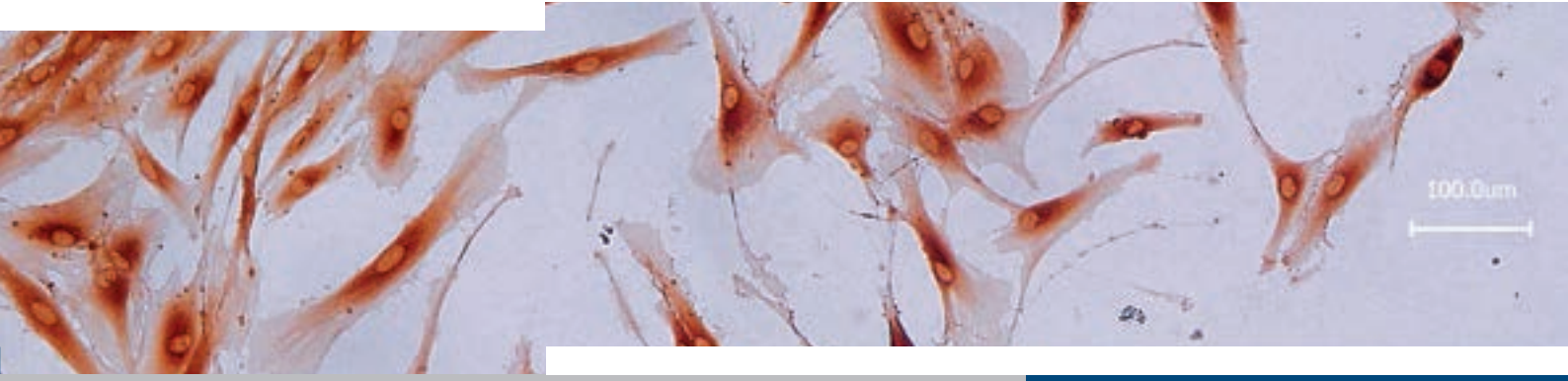
Source: Research Center Jülich, J. Marienhagen

Helmholtz centers and universities develop innovative biological systems

The ”Helmholtz Initiative on Synthetic Biology“ is the first national research network on synthetic biology in Germany. Here, researchers from the Helmholtz centers in Heidelberg, Karlsruhe, Jülich, München and Braunschweig closely collaborate with scientists at the Universities in Heidelberg and Freiburg. The research program is comprised of technology platforms and application projects in the research areas Health and Key Technologies, as well as of two community projects, aiming to promote the development of synthetic biology in Germany to an efficient and responsible research area.

Research within the technology platforms ranges from projects in cancer research, virology, biosensors and enzymology to polymer chemistry and mathematics. The parts and devices developed within these projects will be applied in interdisciplinary application projects.

Scientists at Heidelberg University and the Helmholtz Zentrum München, for example, are developing designer vehicles based on naturally occurring viruses for targeted gene therapy. The combination of surface proteins from natural viral isolates with synthetic sequences should enable a specific therapy of selected tissue and cell types, for example in pancreatic cancer or viral infectious diseases. One project at the Research Center Jülich is focusing on modular synthetic enzymatic cascades and their integration into microbial cell factories. These should enable the utilization of alternative carbon and energy sources or the synthesis of optically active molecules, which can be used as pharmaceuticals, nutritional additives or fine chemicals. Innovative biologically function-



Functionalized biohybride polymers can be used as 3D matrices for the differentiation of stem cells. Shown here are human fibroblasts grown on a stimulus-sensitive hydrogel.

Source: Universität Freiburg, R. Gübeli, W. Weber

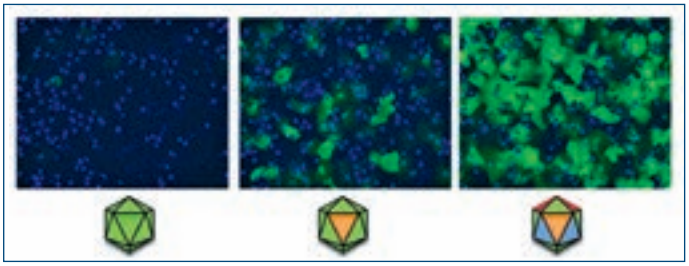
alized polymers are developed at Freiburg University. Materials with tunable features, such as biological and mechanical properties, are functionalized by the integration of synthetic biological switches and can thus be applied by scientists at the Karlsruhe Institute of Technology (KIT) as 3D matrices for the targeted differentiation of neuronal stem cells.

Responsible research includes societal notions

Apart from the chances envisioned by synthetic biologists, there is also an ongoing national and international discussion on potential risks. These risks are anticipated in the area of misuse ("biosecurity") and in potential hazards to human health and environment ("biosafety") as well as in societal and economical risks (König *et al.*, 2012) and traditional ideas of life (Boldt and Müller, 2008). To address these issues, a separate societal research project within the initiative is analyzing ethical and social aspects of synthetic biology and developing a concept for a responsible governance of this emerging discipline.

To strengthen the scientific community of synthetic biologists in Germany, researchers within the initiative are establishing a central repository for biological parts (Helmholtz-Repository of BioParts, HeRBi). The availability of new, standardized biological parts in an open access database shall provide a "molecular toolbox" for synthetic biologists and thus significantly support scientific progress in synthetic biology in Germany.

Improved infectivity of synthetic designer vehicles



Top: Depicted are infected liver cells, in which strength of infection with the target gene is visualized by expression of a green fluorescent protein.
Down: The combination of naturally occurring (green) and synthetic (blue, orange, red) viral surface proteins should improve efficiency and specificity in gene therapy applications.

Source: Heidelberg University, K. Börner, D. Grimm

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EUROPEAN ZEBRAFISH RESOURCE CENTER OPENED AT KIT

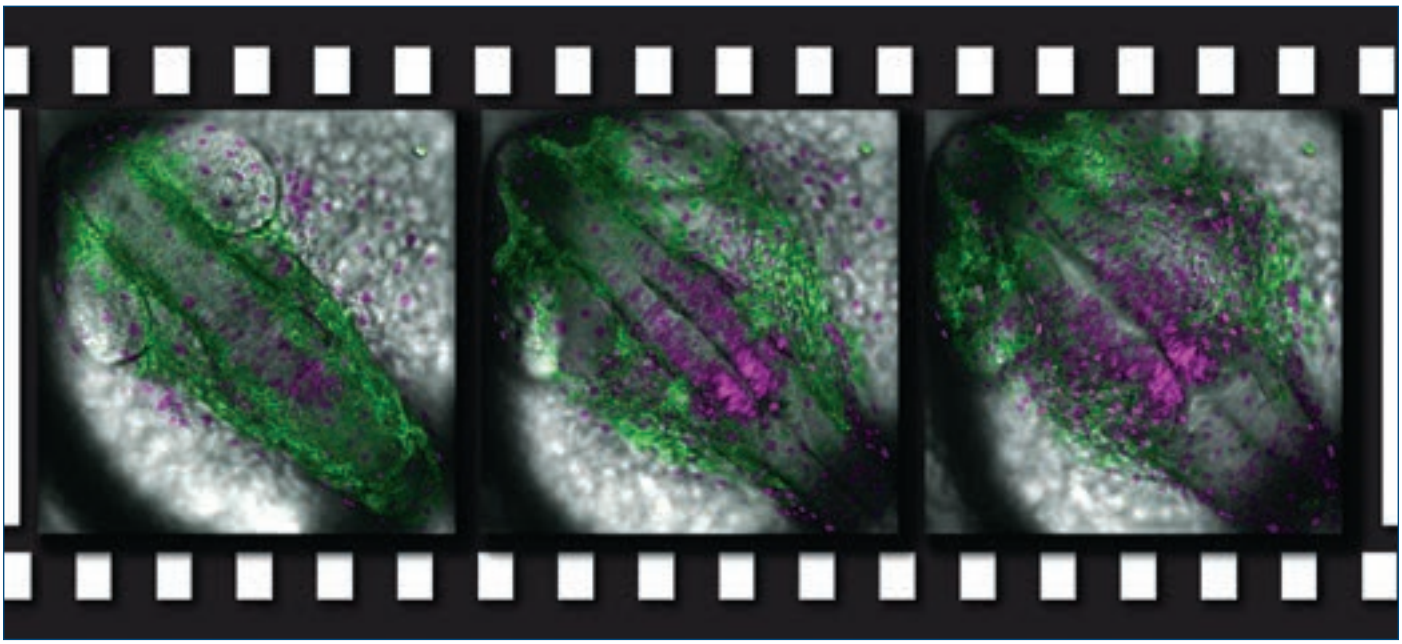
Zebrafish share the most important organ systems with humans. This makes them an ideal model organisms to study the causes of cancer and heart diseases. This research requires a vast variety of different zebrafish lines. In July 2012, the first central repository for such fish lines in Europe, the European Zebrafish Resource Center (EZRC), has been opened at the Karlsruhe Institute of Technology (KIT). The center is funded by the Biointerfaces program of the Helmholtz Association and the Klaus Tschira Foundation, which supports the project with 1.5 million Euros for 3 years.

“Zebrafish are robust, small and reproduce rapidly. At the same time, as vertebrates, they share most important organ systems with humans. These characteristics make them ideal model organisms for biomedical research” explains Professor Uwe Strähle, director of the Institute of Toxicology and Genetics, which runs the European Zebrafish Resource Center at KIT‘s Campus North. “A damaged spinal cord, heart or kidney injuries or destroyed optical nerves are self-repaired – and recover to full organ functionality.”

Additionally, zebrafish eggs are transparent and develop outside of the mother’s body: studies on the development of embryos, the transparent larvae or even single cells are thus possible without damaging the adult animals. Such experiments can substitute for many animal experiments in mice or rats. Fish are very well suited not only for studying the causes of cancer, heart diseases and behavioral disorders, but also for evaluating potential drugs. During the past years, laboratories in Europe alone have generated thousands of different zebrafish lines: each carries either a specific change of the genetic material (mutation), which can serve as a model for a certain disease, or a fluorescent marker labeling a particular tissue.

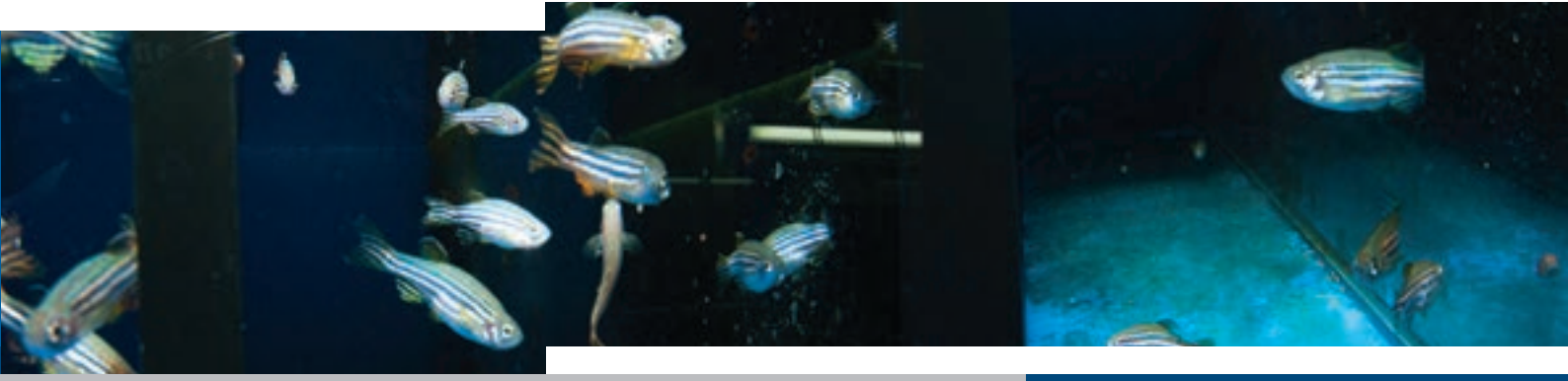
The demand for a much needed central archive for keeping and distributing fish will in future be met by the EZRC. The center has

Confocal time-lapse microscopy of a live zebrafish embryo at the age of 16 to 24 hours after fertilisation



Green fluorescent cells (neural crest cells) migrate from the neural tube and develop into numerous structures (cartilage, pigment cells, nervous system, and so on). A group of cells in the later brain shows high activity of the Wnt signaling molecule, visualized using a fluorescent label (purple).

Sorce: EZRC,KIT



Zebrafish are important model organisms for biomedical research. They are suitable for studying fundamental principles of biology, which also play a role in cardiovascular diseases and cancers.

Source: EZRC,KIT

more than 3,000 aquariums for keeping live fish and freezers for storage of more than 80,000 sperm samples at its disposal. Of particular importance for the acquisition of new fish lines is the separate quarantine area, where new fish lines are being imported for long-term storage. Only authorized personnel adhering to strict hygienic regulations have access to the quarantine. All equipment within the quarantine area is cleaned, disinfected and remains within the facility. External fish lines are only accepted as surface-disinfected eggs and with comprehensive documentation of the source laboratory, legal status, genotype and phenotypic identifying features. After an initial microscopic examination, all embryos are raised, mated and their identified offspring are then transferred to the core facility as surface-disinfected eggs.

The EZRC is also the first screening center for zebrafish worldwide: the facility grants guest scientists access to its collection of fish lines for systematic analyses and provides technologies like high-throughput synthesis of lead compounds and genome sequencing, as well as robotics and software for sample handling, microscopy and image analysis. The EZRC is also a central hub of ZF-HEALTH, a recently launched cooperation project within the 7th Framework program of the European Commission.

Zebrafish lines with diverse genetic mutations are kept in more than 3,000 aquaria at the EZRC

To provide healthy conditions for the animals, aquaria are continuously supplied with freshwater and all important parameters, such as pH, temperature and water supply, are electronically monitored.

Source: EZRC,KIT

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quantification and prediction of cellular processes using Insilico Cells™

Company profile
Insilico Biotechnology AG

by Bettina Stahnke

For which groups of patients will a new drug be effective? Which individual predispositions can be expected to produce side effects? How can animal experiments be replaced? How do changes in culture medium or process control affect the productivity of cells in biotechnological production processes? Questions like these arise every day in the pharmaceuticals and biotechnology sector. Insilico Biotechnology supports innovative life science enterprises in finding answers to such questions with the aid of computer-assisted predictions.

The pressure to bring new products to market as quickly as possible, while keeping development risks and costs as low as possible, is an increasing challenge to companies in the pharmaceutical and biotechnology sector. Efforts to optimise fermentation processes or drug effects and safety lead sooner or later to a focus on quantifying and understanding intracellular mechanisms.

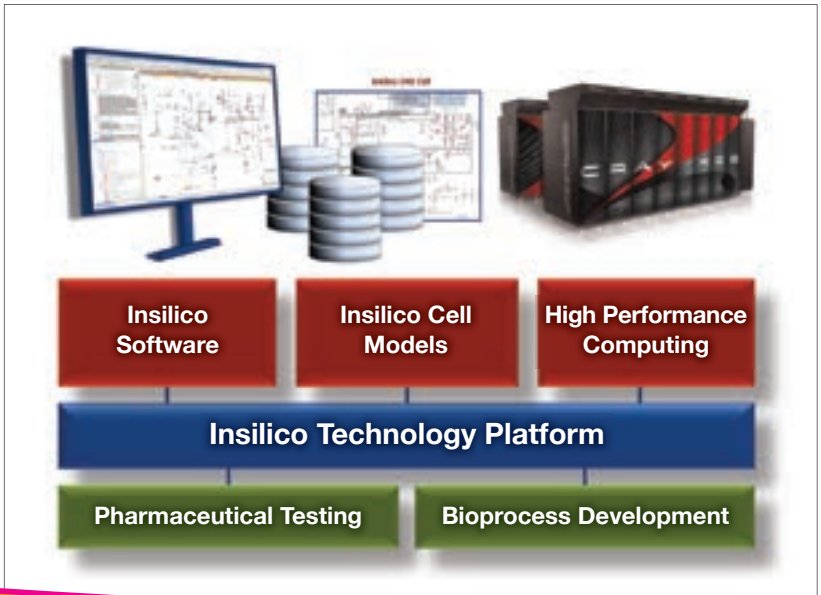
Effective and safe drugs – but how?

In the pharmaceutical industry, the risk of having to abandon a potential drug due to inefficacy or unacceptable side effects observed only in late-stage clinical trials is still high, although several hundred million euros will usually have been invested in development by that time – in this case to no avail.

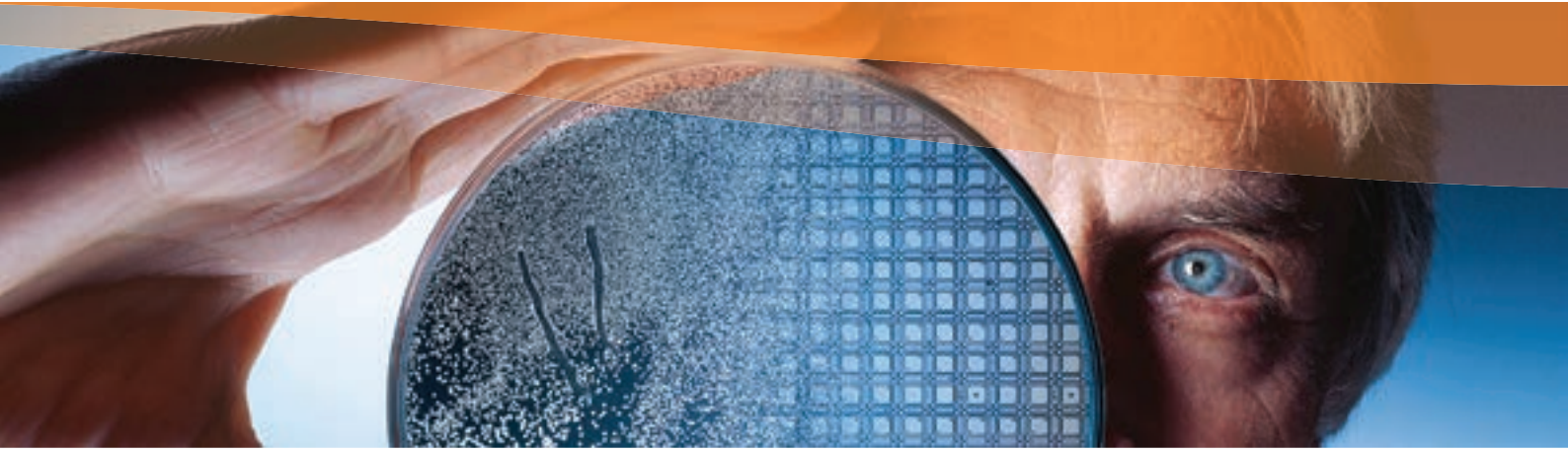
It is therefore desirable to estimate the expected effects of a drug at an early stage, if possible before embarking on expensive clinical studies. To this end, it is necessary to expand preclinical tests in which the differences between different patient groups can also be adequately represented. However, primary cell material from a sufficient number of different patients is often unavailable.

The preclinical phase usually involves experiments on animals, but these are of limited suitability for estimating effects in humans and do not take differences between individual patients into account. Moreover, the social acceptability of animal experiments is still low.

Insilico Technology Platform



To find answers to questions arising from drug development and biotechnology, thousands of biochemical reactions are linked together in computer-assisted cell models known as Insilico Cells™. If required, Insilico Cells™ can be interconnected to form organ and whole-body models. Access to the highly parallel high-performance computer Hermit, one of the fastest computer clusters in Europe, makes light work of the large number and ultra-complexity of calculations performed. (Photo: Cray Inc.).



(Photo: Insilico Biotechnology AG)

Altogether, a respectable range of testing methods is available for the multiplicity of medical questions. However, these methods are of limited validity, and the handling and comprehensive, interconnected interpretation of the resulting data sets is a highly complex task that can hardly be managed without the help of computer-based methods.

High quantities of insulin & co. – but where from?

The same applies to the biotechnological production of complex compounds. Nowadays, the preferred method of producing industrially used enzymes and therapeutically active ingredients such as insulin is in living organisms. Depending on the required product, bacterial expression systems such as *Escherichia coli* or *Bacillus subtilis* as well as cells from higher organisms such as yeasts or mammalian cells are used. Every production process offers a large number of starting points for optimisation. However, the implementation and testing of changes in media composition or feeding strategy, either individually or by statistical design of experiments (DoE), for example, requires a high level of experimental resources and a great deal of patience.

In silico approaches provide answers

This is where Stuttgart-based Insilico Biotechnology AG comes in, by shifting the testing of a wide variety of cellular scenarios from the in vitro and in vivo level to the in silico level, in other words, the level of computer-assisted modelling and simulation. Thousands of biochemical reactions and their mathematical descriptions are collated and linked together to form reaction networks, the Insilico Cells™. The spectrum of pathways taken into account covers metabolic reactions, gene regulation reactions and signal transduction cascades.

After customising and verifying the Insilico Cells™ by implementing the customer's measurement data, millions of different cellular scenarios can be tested systematically with the help of high-performance computing and proprietary software. This enables not only the quantification but also a prediction of the effects of potential changes. The desired and undesired effects to be expected can then be weighed up against each other and Insilico can make appropriate recommendations.

Increasing product yield and productivity in biotechnological production

For example, a production process involving CHO cells was optimised *in silico* with the result that the final product titre was increased by 50% using the ideal media composition ascertained by Insilico.

Further projects included the design of complete cell lines and bacterial production strains by rearranging known metabolic pathways as well as adjustment of culture media for especially promising individual clones. Conversely, clones can be selected based on process guidelines for platform processes, in which the process steps and media used are mostly standardised. Thanks to Insilico's technology, all of these requirements can be met.

Improving the efficacy and safety of drugs

In the pharmaceutical sector, Insilico supports the development process from target validation, i.e. the process of examining the usefulness of a possible target molecule for a new drug, through to preclinical and early clinical characterisation of the drug candidate.

One example for this is estimating the potential effects of changes in cellular signal transduction, which can be relevant in the context of combination therapies for the treatment of cancers. The effects of these changes on cellular metabolism were revealed and quantified by Insilico and its partners.

Insilico Biotechnology is based since the end of 2010 in the Stuttgart Engineering Park (STEP) (Photos: Insilico Biotechnology AG).

In addition, the ability to interconnect respective Insilico Cells™ into organ and whole-body models, and to differentially consider different patient groups, has made it possible to make advanced predictions of the efficacy and safety of a drug candidate, which contributes to making the right decisions at an early stage in the drug development process.

Equipped for the future

With these approaches, Insilico has bridged the gap between the fundamentals of systems biology and industrial application. Targeted prioritisation of active ingredients, experiments and optimisation steps makes it possible to rationalise development processes, thereby helping to shorten them. As a result, Insilico will continue to make an important contribution to technological progress and innovation in the pharmaceutical and biotechnology sector.

Insilico Biotechnology AG in brief:

Insilico Biotechnology AG, a privately held enterprise based in Stuttgart, Germany, is a market leader for solutions and software around the simulation of living cells.

When set up in 2001, the company was a global pioneer in the field of applied systems biology, a business idea that has won recognition with the award of the Start-up Prize of the Otto Beisheim Foundation and the Frost & Sullivan Award for Excellence in Technology, along with nomination by the European Commission for the European Information Society Technologies Prize. While the company's early years were mainly characterised by publicly funded and industrial research projects, it has actively offered services for the biotechnology industry since 2005.

Nowadays, an interdisciplinary team of around 20 experts develops solutions for efficient production of biotechnological products and for testing of pharmaceuticals with the help of high-performance computing and proprietary software.

Insilico technology reduces the duration, risks and costs of development processes for globally leading companies in the chemicals and pharmaceuticals industry.

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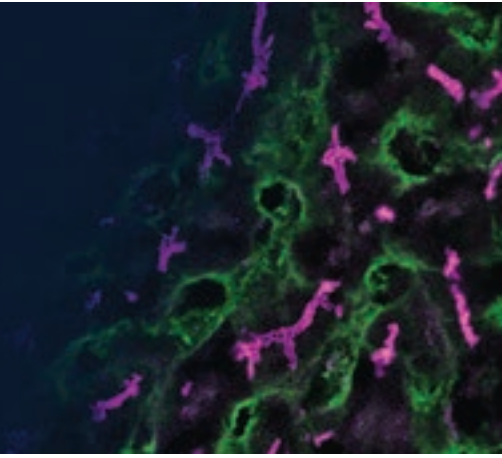
from endosome biogenesis to liver physiology



A multiscale analysis of the small GTPase Rab5

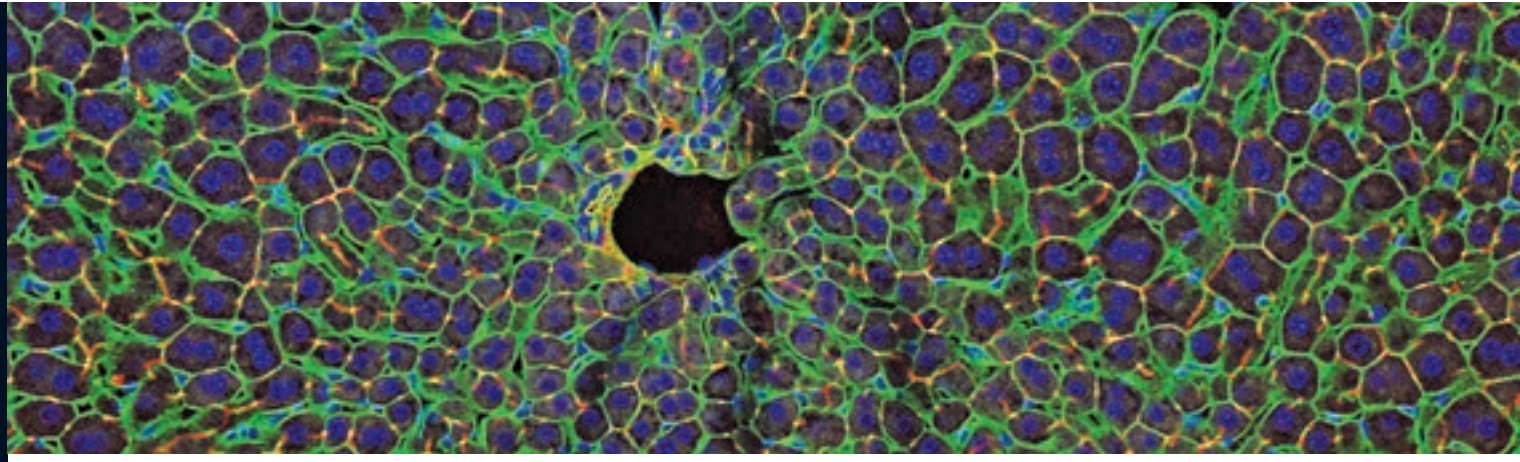
by Anja Zeigerer, Jerome Gilleron, Yannis Kalaidzidis and Marino Zerial

The aim of systems biology is to understand the behaviour of complex biological systems (such as organs or organisms) based on the design principles and properties of their components. That means understanding how molecules assemble to form organelles, and how these organelles contribute towards the functions of cells, tissues, organs and the organism as a whole. Researchers in the Virtual Liver Network funded by the German Federal Ministry for Education and Research (BMBF) succeeded in integrating biological information from the molecular to the organ level by studying the role of small GTPase Rab5 in mouse liver. This study is not only highly significant for understanding the complexity of the endosomal system, but simultaneously provides new approaches to therapeutic strategies for combating metabolic diseases such as diabetes and hypercholesterolaemia in humans.

The liver fulfils a number of vital tasks, such as controlling glucose and lipid metabolism, detoxification of the body and producing bile. In order to perform its functions, the liver cells (hepatocytes) must absorb and release nutrients and receive and process signals. To carry out these processes, the cell has a dynamic network of hundreds of small organelles known as endosomes that play a central role in distributing the internalized substances and in transmitting signals. Signalling molecules and receptors are absorbed into the cell by invagination of the cell surface, where they form small transport vesicles that fuse with early endosomes. Following internalization, the absorbed (endocyted) substances are either transported back to the plasma membrane or degraded in lysosomes. Problems in these transport and sorting steps affect the equilibrium (homeostasis) within the cell and tissue and can lead to serious conditions such as high cholesterol levels (Goldstein and Brown, 2001).

At the molecular level, early endosomes are defined by the presence of the small GTPase Rab5, which together with its 40-plus effector molecules controls the fusion, motility and maturation of endosomes. Rab5 plays a key role in the recruitment and function of the endosomal machinery (Christoforidis *et al.*, 1999). Previously, researchers in the HepatoSys Consortium (forerunner project of the Virtual Liver Network) used biochemical reconstitution experiments to produce synthetic endosomes together with their molecular machinery, and were able to show that Rab5 *in vitro* is indispensable and sufficient for endosome function (Ohya *et al.*, 2009). However, it had still not been possible to prove the significance of Rab5 for the formation of endosomes *in vivo*.

To solve this problem, we first developed a mathematical model based on what was known so far about the biochemical properties of Rab5. The model calculates the dependency of endosome formation based on the concentration of Rab5 and delivers possible predictions. Some predictions suggested an increase in the number of endosomes as the Rab5 concentration decreases, while others suggested a reduction in endosomes. In order to test these different options, we performed a knockdown of the three Rab5 genes in the liver of adult mice and tested the effect of a progressive reduction in Rab5 concentration on the number of endosomes. This was done using state-of-the-art RNA interference technology (RNAi) *in vivo* by injecting lipid nanoparticles (LNPs). This technique, originally developed for therapeutic purposes, permits an efficient (up to 85%) hepatocyte-specific and reversible knockdown of up to ten different genes (Akinc *et al.*, 2008). We were thus able to reduce the expression of Rab5 specifically in the liver and to analyse the consequences of Rab5 depletion on the subcellular, cellular, tissue and organ level. This multiscale analysis enabled us to establish Rab5 as a master regulator of endosome biogenesis *in vivo* and to show the significance of endosomes for hepatocyte polarity and liver metabolism (Fig. 1).

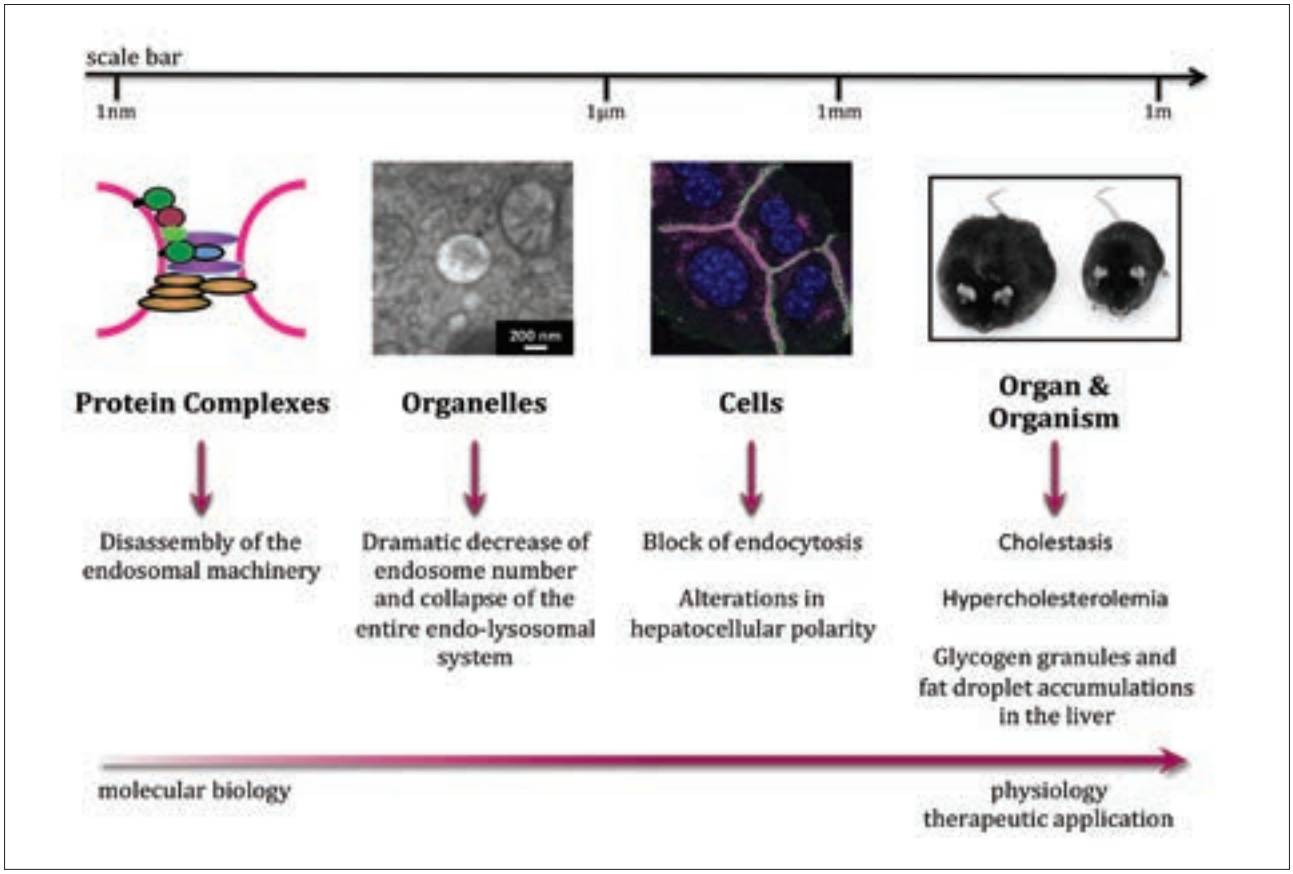


Knockdown of Rab5 leads to a loss of the endolysosomal system and defects in polarised membrane trafficking. Distribution of an apical marker CD13 (red) in control liver tissue. The actin staining in green shows cell boundaries, while nuclei within a cell are shown in blue (Photo: Anja Zeigerer).

Rab5 is essential for the formation of endosomes

Astonishingly, a 50% reduction in the Rab5 concentration three days after injecting the RNAi liposomes had no effect on the number of endosomes. This shows that the endocytic system is very robust and able to tolerate major fluctuations in the amount of Rab5. In contrast, five days after administering the LNPs, corresponding to an 85% reduction of Rab5 levels dramatic effects were observed. The number of early and late endosomes and lysosomes decreased drastically (by up to 80%). This result clearly establishes Rab5 as the master regulator in the formation of endosomes and shows that there is a critical threshold value of Rab5 below which the entire endolysosomal system collapses. The data obtained confirms one scenario in our mathematical model, in which a reduction in the number of endosomes was predicted depending on the Rab5 concentration. Moreover, the simultaneous loss of lysosomes indicates that these organelles are formed partly from endosomal material. Using this observation as a starting

Figure 1: Diagram of a multiscale analysis



Multiscale analysis can be used to integrate knowledge from different biological levels, starting from protein complexes through to the function of cells, organs and the physiology of the entire organism. RNAi technology enables *in vivo* knockdown of a specific protein in order to investigate the depletion impact from the molecular to the organ level (Diagram: Anja Zeigerer and Jerome Gilleron).

Prof. Dr. Marino Zerial, Dr. Anja Zeigerer and Prof. Dr. Yannis Kalaidzidis (from left to right) developed a multiscale analysis that enables the analysis from the molecular level to the organism. Co-author Dr. Jerome Gilleron is not in the picture (Photo: F. Friedrich).

point, it is now possible to explore the role of endosomes in the establishment of hepatocyte polarity, liver function and the integrity of the organism as a whole.

Endocytosis is necessary for the maintenance of hepatocyte polarity

Hepatocytes are polarised cells with differently structured and functional cell membranes (Fig. 2, left-hand diagram). The basal membrane is facing the blood, while the membrane bordering on the bile duct is known as the apical cell membrane. These different membranes are characterized by the localisation of different surface molecules such as receptors and transporters, which fulfil special tasks and functions of the liver. A faulty arrangement of transporters at the apical or basal membranes can lead to diseases of the liver and bile ducts. Since the endosomal system plays a central role in the proper sorting of molecules and signalling substances, we postulated that it is also required for the maintenance of hepatocyte polarity. Hepatocyte-specific removal of Rab5 gave us the opportunity to investigate this hypothesis *in vivo*. We were able to show that the multi-drug-resistant associated protein 2 (MRP2) does not require any endosomal sorting stage and is transported directly to the apical membrane, confirming a previous hypothesis postulated from 2004 (Wang and Boyer, 2004). In contrast, other apical proteins such as DPPIV (Dipeptidyl-Peptidase-4) and BSEP (Bile Salt Export Pump) need an intact endomembrane system in order to reach the correct destination (Fig. 2, right-hand diagram). These results showed for the first time the significance of endosomes in maintaining cell polarity in the liver.

The loss of Rab5 in the liver leads to severe metabolic defects

Impaired function of a central metabolic organ such as the liver often triggers metabolic diseases such as biliary stasis, insulin resistance in diabetes, or high cholesterol levels. The reduction in Rab5 expression led as expected to a rapid decrease of LDL (low-density lipoprotein) absorption by the liver cells and resulted in a tenfold increase in serum cholesterol. This phenotype corresponds to the pathological symptoms of hypercholesterolaemia (Goldstein and Brown, 2001). In addition, the disturbance of cell polarity in liver due to incorrect distribution of BSEP led to the onset of biliary stasis (cholestasis). Surprisingly, the reduction in Rab5 concentration and the subsequent loss of endosomes caused glucose storage in the form of glycogen in hepatocytes and led to the formation of intracellular fat droplets (Fig. 2, right-hand diagram). These results point to a previously unknown function of endosomes in the regulation of sugar and fat metabolism, the mechanism of which requires further investigation.

Conclusion and outlook

Our findings show first that Rab5 is indispensable for the formation of the entire endosomal system consisting of more than 1,000 different proteins. Rab5 knockdown has fatal consequences for the maintenance of cell polarity in the liver and the entire liver metabolism. Some of the perturbations triggered could certainly be foreseen, while others, such as the connection between endocytosis and cellular liver metabolism, were unpredictable.

Second, *in vivo* RNAi technology enables the multiscale analysis of specific genes in the liver and thus the integration of knowledge gained at different biological levels. This integration permits us to draw conclusions about the functions of the different levels and to create a physiological link between these modules. Based on this discovery, we now know that changes in the endosomal system have extreme consequences for liver metabolism and can generate pathological metabolic disturbances.

Third, the technology of *in vivo* RNAi knockdown of specific genes in the liver provides the opportunity to use this strategy for therapeutic purposes, such as combatting diabetes and hypercholesterolaemia. These results are therefore an important starting point for possible new therapies to treat metabolic disorders in humans (Zeigerer *et al.*, 2012).

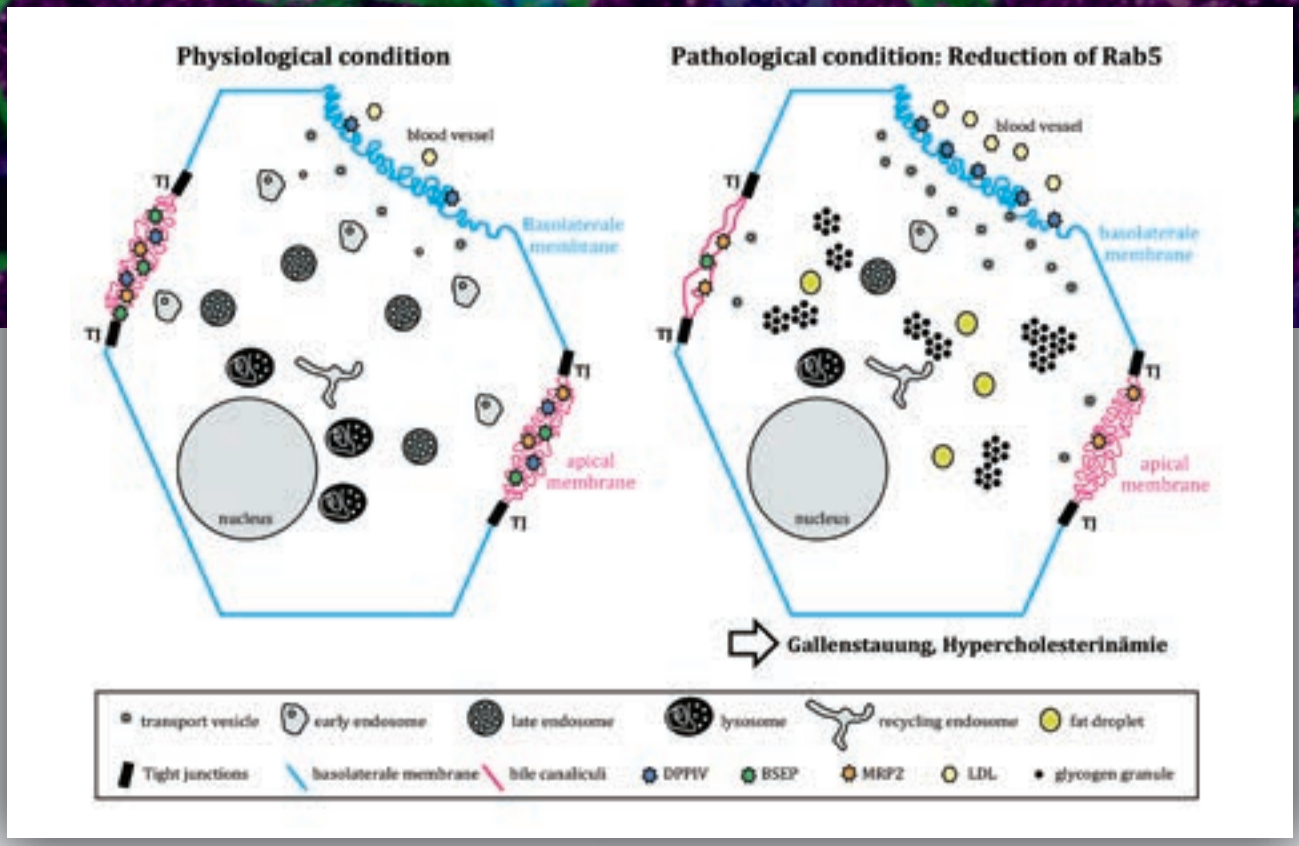


Figure 2: Specific reduction of Rab5 in the liver *in vivo* permits the analysis of the role of Rab5 at the molecular, cellular and organism level (Diagram: Jerome Gilleron and Anja Zeigerer).

The research project in brief:

The Virtual Liver Network is a nationwide initiative funded by the German Federal Ministry for Education and Research (BMBF). It comprises 70 research groups spread throughout Germany. To support its own work, the Network develops ties with other research groups and international initiatives. Virtual Liver aspires to a dynamic model that, while not completely simulating the physiology, morphology and function of the human liver, nonetheless maps it in the form of a model. In doing so, quantitative data from all stages of the liver's organisation are integrated into the model. The programme director is Adriano Henney (see also *"How much systems biology does the Virtual Liver need?"* on page 68).

www.virtual-liver.de

Research group website:

http://www.mpi-cbg.de/research/research-groups/marino-zerial.html

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fanci: functional analysis of non-coding RNAs in living cells

A report on the success of the BMBF-funded SysTec project

by Holger Erfle

Due to the use of modern high-throughput technologies, a steadily growing number of different genomes is being sequenced and analysed. Interestingly, it appears that in a mammalian cell, for instance, only about 1.5% to 2% of the DNA open reading frames are ultimately translated into proteins.

A significantly higher proportion, about 25%, fulfils the prerequisites for being transcribed into RNA. However, these non-coding RNAs (ncRNAs) do not serve as the basis for translation into proteins. Apparently, their job is rather to regulate cellular processes. As more and more ncRNAs with this type of function are being discovered, they may form a highly complex regulatory cycle.

Naturally, this raises the question whether ncRNAs are as important as proteins for the regulation of vital cellular and organismal functions. By way of example, partners in the joint project "Functional Analysis of Non-Coding RNAs in Living Cells" (FANCI), part of the "SysTec – New Methods in Systems Biology" programme funded by the German Federal Ministry for Education and Research (BMBF), examined the influence of non-coding RNAs on the secretory membrane transport pathway. The main focus of the project is not just on this important biological question. The work is distinguished especially by the development of methods and technologies for functional analysis that forms the basis of a planned spin-off.

Since the start of the project in September 2009, some important results have been achieved. With an unexpectedly high number of 44 miRNAs (a sub-class of ncRNAs) and also of

Figure 1: High-density spotting robot from Graffinity Pharmaceuticals GmbH for printing identical high-density cell arrays

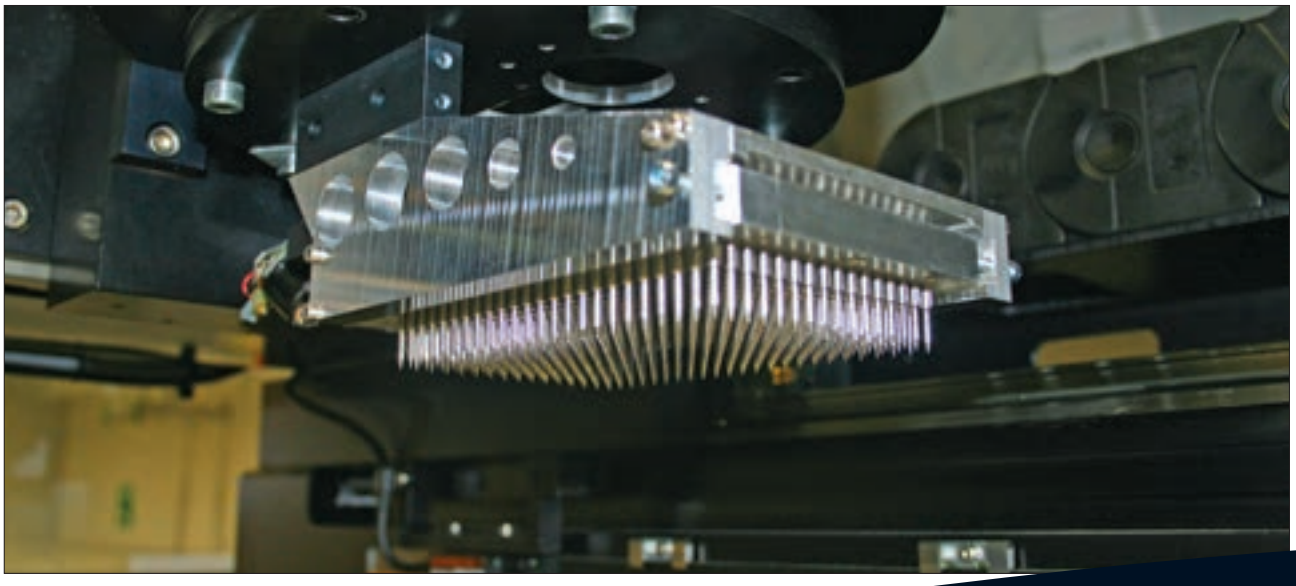

Photo: Jürgen Reymann

Figure 2: Leica TCS SP5 automatic confocal microscope with incubation chamber for live-cell imaging (Photo: Nina Beil).

selected pseudogenes, we found marked effects on membrane transport, one of the most important cellular processes. Malfunctions in the secretory membrane transport pathway can lead to enormous damage to health, such as the emergence of cancer. An aberrant regulation resulting from the malfunctioning of ncRNAs seems therefore to be of critical significance. This renders the new kinds of inhibitors developed in the project, based on 2'-OMe RNA or phosphorothioate DNA, which can for example be used to combat low abundant ncRNA with high specificity.

The development of a whole genome cell array provides the world's first platform with almost 25,000 physically separated reaction troughs in an area the size of a 96-hole plate. Special coatings and treatments make it possible to transfect and analyse cells with a specific ncRNA knock-down in every trough. In order to introduce these few nanolitres in the required quantity in a very short time, a high-density spotting robot was developed (Fig. 1) that works with 384 needles in parallel and image processing-assisted control.

Holger Erfle heads the ViroQuant-Cellnetworks RNAi Screening Facility at the BioQuant Center (Heidelberg University) (Photo: Hendrik Schröder).

The images from the automatic wide field and confocal laser scanning microscopes (Fig. 2) are examined for relevant phenotypes using innovative image processing algorithms. This makes it possible to ascertain cellular to sub-cellular phenotypes (e.g. Golgi fragmentation) for living cells on the array automatically and highly accurately by means of combined segmentation and classification methods.

The results were turned into a mathematical model by means of novel statistical methods that enable further-reaching analysis of the microscopy data. This makes it possible to explain central steps in the control of membrane transport. The results of experiments and literature research are stored in a database that will be publicly available in future.

The research project in brief:

The FANCI project (FANCI = functional analysis of non-coding RNAs in living cells) is running for a four-year term (2009–13). The grant recipient is Heidelberg University. Project Leader: Dr. Holger Erfle, Heidelberg University. Cooperation partners: PD Dr. Karl Rohr, Heidelberg University, Dr. Reinhard Schneider, LCSB, University of Luxembourg , Prof. Lars Kaderali, TU Dresden, Dr. Vytaute Starkuviene, Heidelberg University, Dr. Andriy Mokhir, Heidelberg University, Dr. Benedikt Busse, zell-kontakt GmbH, Nörten-Hardenberg, and Klaus Burkert, Graffinity Pharmaceuticals GmbH, Heidelberg.

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cellular self-cannibalism – the interplay of autophagy and apoptosis

Portrait of Anne Hamacher-Brady

by Claudia Eberhard-Metzger

Cells ensure that they are in good working order by "running" a subcellular self-cannibalism program known as autophagy. If the autophagy program contains defects, for example running too fast or too slow, cells may age more quickly or become ill. A new independent research group at the BioQuant Center at the University of Heidelberg is investigating this fundamental cellular phenomenon in order to gain insights into disease mechanisms, for instance cancer, at cellular and organismal levels.

The organisms aiding Dr. Anne Hamacher-Brady to understand how life is organized are very small. *Caenorhabditis elegans* measures barely one millimeter and is a "star" in the world of biology since the Nobel Laureate Sydney Brenner introduced these transparent nematodes as model system in the 1960s. To date, *C. elegans* has aided the cell biology research community to win three Nobel prizes and provided incalculable insights into the complex, still not fully-resolved, life of cells. Dr. Hamacher-Brady aims to use these nematodes, which consist of circa 1000 cells, to gain a better grasp of autophagy, a process of microscopic self-digestion of the cell.

Autophagy and the closely-related phenomenon of programmed cell death, or apoptosis, have fascinated the young biologist since her undergraduate studies at the RWTH Aachen. In 2002 she had the opportunity of spending a research semester studying molecular cell physiology at the Free University of Amsterdam. She subsequently completed her degree there with a thesis on the role played by mitochondria, the powerhouses of the cell, when myocardial (heart) cells die by apoptosis as a result of oxygen and nutrient deficiency during myocardial infarction. As a doctoral student in the Department of Molecular and Experimental Medicine at the Scripps Research Institute in La Jolla, California, Dr. Hamacher-Brady investigated the relationship and roles for apoptosis and autophagy in the damage caused to heart cells by infarction.

Dr. Anne Hamacher-Brady heads the BMBF e:Bio Research Group 'Lysosomal Systems Biology' at the DKFZ Heidelberg (Image: A. Hamacher-Brady).

This work in the then nascent field of molecular autophagy research led her to the basic discovery that autophagy aids damaged myocardial cells to survive. In October 2006 Dr. Hamacher-Brady returned to Germany and since then has been studying autophagy and apoptosis mechanisms at the German Cancer Research Center (DKFZ) in Heidelberg.

Since March 2012, the biologist is leading the independent research group 'Lysosomal Systems Biology', for which she received 1.32 million euros in funding from Germany's Federal Ministry of Education and Research (BMBF). "Our goal is to view the molecular processes that influence programmed cell death in their entire context," explains Dr. Hamacher-Brady. The model systems used by her group are cultured cancer cells, from which basic findings are then eventually inves-

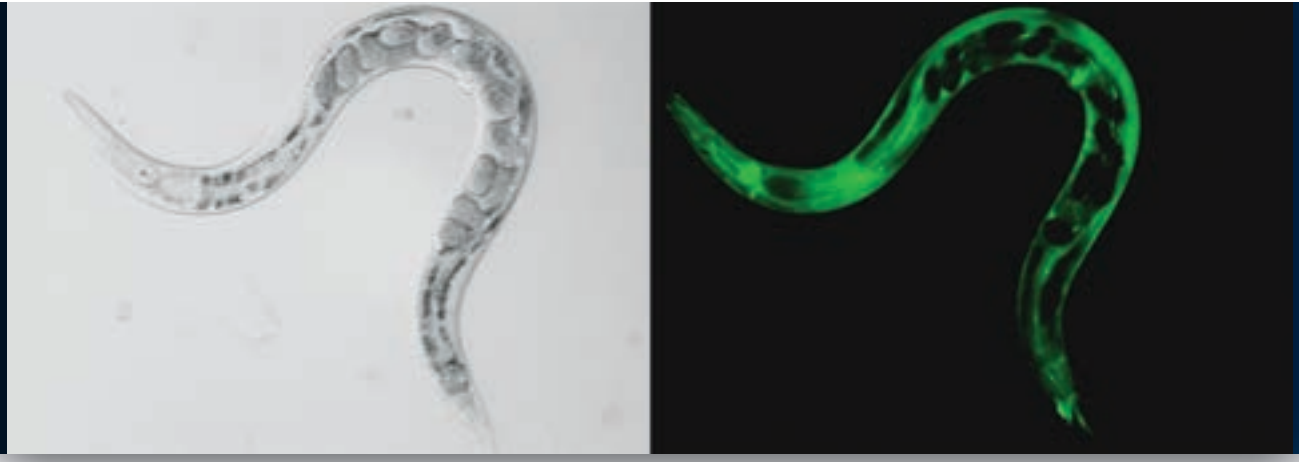


Figure 1: *C. elegans* expressing a green fluorescent protein (GFP)-fused version of the autophagosome marker LGG1. Upon autophagy induction, cytoplasmic LGG1-GFP is incorporated into autophagosomal membranes. The image shows an adult *C. elegans* hermaphrodite. Left: transmitted light microscopy, right: fluorescence image of LGG1-GFP (Image: A. Hamacher-Brady).

tigated in nematodes, in order to understand the processes at the level of the organism. "*C. elegans* provides the ideal balance between complexity and simplicity in terms of both its genomic and its physiologic design," says Dr. Hamacher-Brady.

In the lab situated adjacent to the office she opens the door of an incubation cabinet, removing a Petri dish. The tiny organisms on the light-colored background of the Petri dish are barely recognizable by the naked eye, but impressive when seen under the microscope. The tiny worms weave an elegant trail through the field of view. Dr. Hamacher-Brady switches the microscope to fluorescence mode, and the transparent bodies of the tiny, wriggling organisms glow green. "The glow comes from green fluorescent protein targeted to the mitochondria of muscle cells." she explains. *C. elegans* can be altered by means of genetic methods so that fluorescent signals report activities of genes in the signaling pathway of autophagy (Fig. 1). "Such genetic manipulations can be made quickly and the lifespan is counted in days," Hamacher-Brady explains. "That enables us to rapidly test model predictions, and it also makes *C. elegans* the ideal animal model for systems biology."

The systems biology approach of her group, says Dr. Hamacher-Brady, is necessary in order to come to grips with cellular complexities. For a long time, she maintains, the molecular signaling pathways leading to apoptosis or to autophagy were thought to be separate phenomena. However, it is becoming increasingly apparent how closely the two cellular processes interact and undergo intensive crosstalk. Both processes, Dr. Hamacher-Brady explains, involve fundamental signaling programs that share key components, used by cells for self-preservation or, if necessary, for controlled death.

Intracellular self-digestion

Discovered about half a century ago, autophagy was overshadowed for a long time by the phenomenon of apoptosis. While the molecular players of the apoptosis program are now well elucidated, the mysteries of autophagy remain to be unraveled. The term 'autophagy' is derived from Greek roots and means 'self-eating'. Researchers can study this self-cannibalism within the cell under the microscope. Initially, membranes appear in the cell, rapidly growing and then closing to form a double-membrane vesicle. The enclosed cytoplasmic constituents – various macromolecules or cell organelles, for instance a defective mitochondrion (Fig. 2) – are now sequestered inside the vesicle and securely wrapped up by the double-layer membrane.

This membrane structure, referred to as 'autophagosome', delivers the content to another vesicle enclosed by a membrane – the lysosome, which is filled with digestive enzymes. The autophagosome fuses with the lysosome, and the lysosomal enzymes break down the engulfed cell material into component parts. The components serve the cell as material for creating new macromolecules or new organelles. Dr. Hamacher-Brady sums up the current state of our knowledge as follows: "We now know that functional and regulatory proteins are specially marked for autophagic degradation, which means that a vast number of signaling pathways can be selectively regulated by autophagy."

Under normal circumstances, the cell uses autophagy to remove disruptive waste that is produced during the operation of the 'cell factories', such as used or incorrectly folded proteins, dysfunctional organelles, or of dangerous intruders such as viruses or bacteria.

Autophagy is thus not a self-destructive process but rather guarantees a healthy cellular homeostasis. Scientists have now identified about 35 genes that are known to control the molecular apparatus and can produce more or less activity, as required. When a cell encounters a stressful situation, for

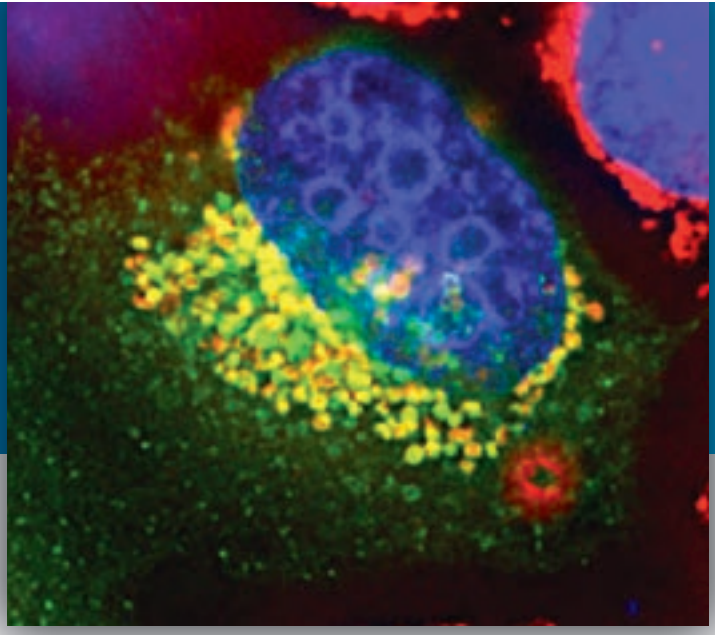


Figure 2: Autophagic degradation of mitochondria, mitophagy, in breast cancer cells. Mitochondria are normally cellular producers of energy, but during apoptosis they can contribute to the programmed cell death. The mitochondrial contribution to cell death is, in turn, strictly controlled by the process of autophagy. The cell nucleus is shown in 'blue', the mitochondria in 'red' and the lysosomes in 'green'. Cellular structures containing both mitochondrial and lysosomal markers, i.e. active mitophagy, appear as 'yellow' (Image: A. Hamacher-Brady).

instance if nutrients become scarce, or too many metabolic end products accumulate or pathogens intrude, the cell steps up its autophagic activity. Autophagy is an instrument that enables the cell to protect itself and survive, but the cell can also use this instrument to destroy itself, say if very severe damage has occurred that turns the cell into a threat for the entire organism. The importance of autophagy as a fundamental process in life is reflected in the fact that it developed early in evolution and is found in both unicellular and multicellular organisms, be it in nematodes or in human beings.

Given the importance of this program, it is hardly any wonder that disease threatens the organism if autophagy malfunctions. Cancer, Parkinson's, Alzheimer's and aging processes are associated with autophagy that runs too fast or too slow or is otherwise defective. "The better we understand the molecular details of autophagy," says Dr. Hamacher-Brady, "the more specific points of attack can be found, for example for new, selective drugs against cancer and other diseases for which we are seeking improved therapies."

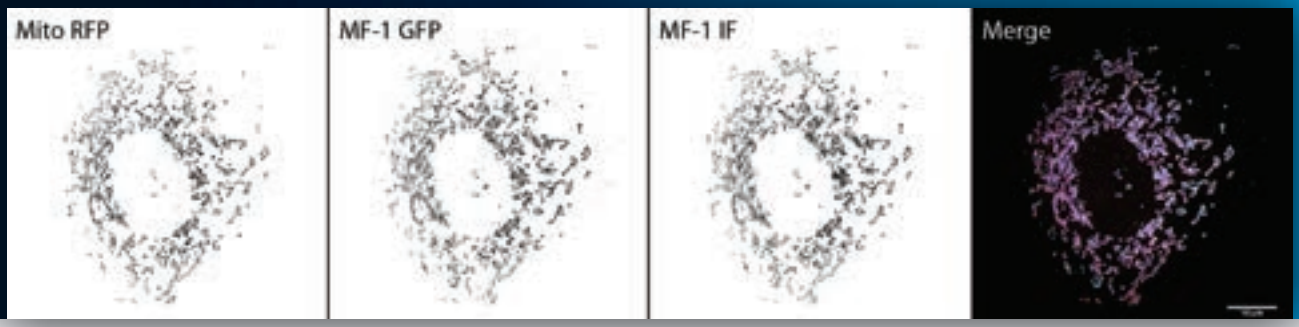
Hanging above Dr. Hamacher-Brady's desk are the electron microscopy images (photomicrographs) of lysosomes, the tiny cell organelles that are filled with digestive enzymes and are so crucial for the life and death of cells. Together with her

BMBF e:Bio Research Group "Lysosomal Systems Biology"

Programmed cell death (PCD) is regulated by the interplay of genetically-defined signaling pathways that are coordinated both spatially and temporally. The understanding of PCD is of central importance, in that its successful execution is the key to cancer therapy. As fundamental discoveries concerning PCD machineries or even new modes of PCD are still being reported, addressing the complexity of PCD signaling is a growing challenge. Systems biology offers tools to utilize such biological complexity, through full data integration and mathematically-derived non-intuitive hypothesis generation.

Intriguingly, PCD undergoes substantial positive and negative regulation by the endo-lysosomal compartment. Our group is dedicated to the elucidation of lysosomal control of PCD in cancer and non-cancer cells, with an emphasis on testing theoretical model predictions, derived from cell culture experiments, in *C. elegans*. Quantitative fluorescence microscopy and biochemical methods are combined with systems biology modeling approaches, and the findings from these experiments are translated to the level of the organism. Primary goals are the systemic identification of regulatory mechanisms governing lysosomal and PCD signaling pathways, and confirmation of *in vivo* functionality. Our research will provide insights into specific mechanisms for optimizing PCD in cancer cells.

A. Hamacher-Brady



Human cervical cancer cells transfected with a red-fluorescent mitochondrial marker (mito RFP) and the green-fluorescent mitochondrial factor (MF-1 GFP). In addition, MF-1 has been visualized by means of immunofluorescence (MF-1 IF). The multicolor image shows the various fluorescent images overlaid (merge) (Image: A. Hamacher-Brady).

husband, Dr. Nathan Brady, who also heads an independent research group at the BioQuant Center, the biologist recently succeeded in demonstrating the role that lysosomes play in cell death induced by artemisinin and its derivative artesunate. Artemisinin is a natural compound extracted from the medicinal plant *Artemisia annua*, or annual wormwood. It has been used in recent decades as a treatment for malaria, but it has apparently also been found to be effective in killing of cancer cells.

The decisive factor for artesunate-induced cell death is to be found in the lysosomes and the iron that they contain. "The iron reacts in the lysosomes with the artemisinin-derivative artesunate," explains Dr. Hamacher-Brady. This gives rise to reactive oxygen species that set in motion signaling events that cause mitochondria to initiate apoptosis. The researchers discovered that the active substance is also capable of interfering with the autophagy program of cancer cells. Artesunate blocks autophagy in the degraded cells, thereby preventing the cancer cells from ensuring their survival. Artesunate is currently being tested in early clinical trials for its suitability as a drug to treat various types of cancer.

The research project in brief:

Project name: BMBF e:Bio Research Group – Systems Biology in Cells and Worms: Modeling of *in vivo* Lysosomal Control of Programmed Cell Death in Cancer (LysoSys). This project is funded by the BMBF initiative "e:Bio – Innovation Competition Systems Biology".

Partners: BioQuant Center of the University of Heidelberg and the German Cancer Research Center.

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Google Scholar profile for Hamacher-Brady:
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system-relevant: ageing of stem cells

The SyStaR Project at Ulm University

by Hans Kestler, Hartmut Geiger and Karin Scharffetter-Kochanek

The research core SyStaR "Molecular Systems Biology of Impaired Stem Cell Function in Regeneration during Ageing" is a systems biology project at Ulm University with the aim of improving our understanding of the processes that lead to the ageing of stem cells. In the long term, the hope is that this will enable scientists to develop new therapies to improve organ function in old age. SyStaR brings together know-how from the fields of systems biology, stem cells, ageing, model organisms, signalling pathways, and geriatric medicine.

Stem cell ageing

As society ages, the study and treatment of age-related diseases becomes increasingly important. One factor that significantly impairs the quality of life in old age is the impaired performance and regenerability of the body's organs. It is generally supposed that the decline in the self-renewal and functioning of adult stem cells and the loss of regenerability of tissue cells in organs are the factors responsible for the ageing of organs. This process affects particularly organs and tissues with high rates of cell division, for instance the hematopoietic system, the skin, and the intestinal epithelium. Certain conditions such as the frequent occurrence of anemia, impaired wound healing, and functional disorders of the intestine in geriatric patients support this hypothesis.

Furthermore, stress reactions in the wake of injury or disease can exacerbate the age-related decline in stem-cell function and the loss of regenerability. For example, old age is one of the principal risk factors for a person developing cirrhosis of the liver as a result of chronic hepatitis. The limitations on stem cell function and the regeneration of tissue cells can be triggered by changes within the cell but may also be changed by environmental factors. For instance, damage to the DNA in the cell may be the reason for ageing processes within the cell but at the same time can trigger changes in the environment. SyStaR team members have shown that changes in the immune system that restrict wound healing may well be among these extracellular reactions.

Experimental data from studies in mice and fruit flies (*Drosophila*) now demonstrate that the ageing of stem cells in particular is triggered by changes in certain regulation networks that were previously important for stem cell development. The Wnt signalling pathway and the notch signalling pathway, for instance, are both associated with a decline in stem cell function that goes hand in hand with advancing age. However, the causes that lead to molecular changes in ageing stem cells and in the environment of stem cells is still only incompletely elucidated. In all likelihood, a number of factors are involved in these processes. The SyStaR project at Ulm University is one of three research cores that are being funded by the GerontoSys Initiative of the German Federal Ministry of Education and Research. Through cooperation with researchers from various disciplines such as medicine, biology, information technology, and mathematics, SyStaR aims to elucidate the causes of molecular stem cell ageing and explore some of the therapy options.

The objective

The main objective of SyStaR is to identify deviations in signalling pathways that restrict the functionality and the regeneration of stem cells in old age. SyStaR has also demonstrated that there are areas in which the proposed studies could have a significant economic impact (Figure 3 shows an overview of the scientific concept underlying SyStaR).

(i) One of the most important goals of the SyStaR Consortium is to use systems biology methods to generate mathematical models of stem cell ageing on the basis of experimental data. With the aid of these integrative models, the scientists can identify changes in signalling pathways that are associated with an age-related decline in stem cell function and the loss of its ability for self-renewal.

Figure 1: The members of the SyStaR Consortium at the kick-off meeting on October 23, 2012 (Photo: Annika Bingmann, Ulm University press office).

(ii) Development of specific therapies to improve the function and regenerability of stem cells. The deterioration of organ function is one of the main problems adversely affecting the quality of life of the elderly. The few existing therapy options are expensive and in any case unable to arrest the decline in organ function. Research into therapies of this kind would herald a breakthrough in modern medicine and would have enormous economic and socio-economic potential.

(iii) Identification of biomarkers that shed light on stem cell regenerability in old age. Such biomarkers will be instrumental in enabling physicians to predict the outcome of invasive treatments and the individual risk of disease progression in elderly patients. Biomarkers that provide information on stem cell function could help medical science develop therapies that are individually tailored to ageing patients. In addition, biomarkers of this kind have enormous economic potential for the development of new drugs. Given the long life span of mammalian models, research on ageing is both expensive and time-consuming. With the aid of robust biomarkers, the positive and negative implications for the function and renewal of stem cells could be determined much more quickly. This would significantly increase the efficiency of such gerontological studies. This fact makes the large-scale projects attractive for companies in the healthcare sector as well.

Methodology

SyStaR is using various model organisms to achieve its goals. An innovative genetic approach known as QTL analysis, which is based on recombinant inbred mice, will be used to identify molecular signalling pathways that regulate stem cell ageing. In addition, SyStaR is using genetically modified mouse mutants and the Drosophila fruit fly in its research program on stem cell ageing as well as cell systems. For the most part, these are human cells and yeast cells. Researchers are employing primary human cells in order to study signalling networks that limit the survival time of human stem cells. In a complementary approach, SyStaR will study yeast cells in order to analyse signalling networks in cell ageing. The SyStaR project members will also deploy their experience in the field of molecular imaging in order to identify protein networks that regulate asymmetric cell division in yeast cells. The knowledge they gain will then enable them to draw conclusions about asymmetric cell division in adult stem cells.

One of the major challenges that ageing research faces is to demonstrate that molecular ageing mechanisms replicated in model organisms are also relevant for ageing in humans. The model systems and approaches employed in SyStaR are very powerful instruments, making it possible to identify universal ageing mechanisms, which are therefore also relevant for human beings. If, for example, we know what the effect is when particular genes are inhibited, we can develop therapeutic strategies based on this knowledge that target the same molecules and signalling pathways by pharmacological means. The interaction of medical science, molecular biology and systems biology is important in this connection. Replicating the systems to be investigated in mathematical models provides the opportunity of testing the effects of interventions in the systems *in silico*, i.e. via computer simulation. With this approach, researchers can identify potential targets for intervention and propose hypotheses on regulatory links, which they can then verify in targeted molecular biology experiments (see Fig. 2). The scientists in the SyStaR project have already demonstrated that targeted molecular biology interventions in target organs of animal models can be harnessed to validate new regulatory mechanisms identified in computer simulations. All these investigations show that the relevant information is in some cases hidden under irrelevant "noise". Stochastic models and statistical data analyses are important tools for validating models and improving the methods.

Perspectives and applications

Within SyStaR, regulatory signalling pathways are linked to ageing-related clinical phenotypes in order to advance practical applications stemming from the findings. These applications take the form of new therapies. SyStaR researchers have

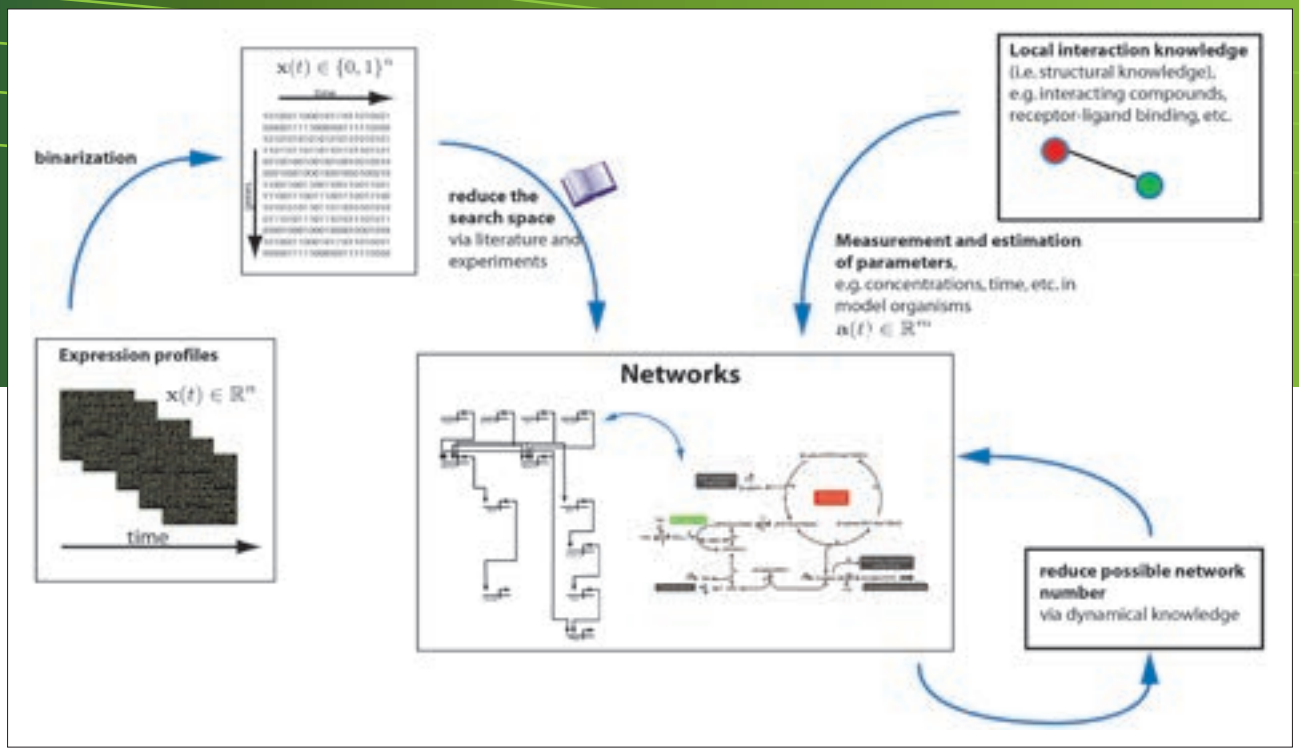

Figure 2: Model development by reconstruction on the basis of gene expression data and by integrating knowledge of local interactions derived from the literature, own experiments, and databases (diagram: Hans A. Kestler).

developed methods that allow them to evaluate the functioning of a signalling pathway in a clinical context in elderly patients. The use of modern geriatric methods for examining elderly patients is of crucial importance in order to prevent certain variables such as handicaps, physical frailty and other unrelated illnesses from being confused with the influence of the signalling pathway the researcher is measuring. Research that SyStaR partners are conducting shows, for instance, that life style factors go hand in hand with an increase in DNA damage in humans that is unrelated to ageing. SyStaR thus provides an innovative platform enabling scientists to exploit network analyses in the clinical field. To achieve this goal, the interdisciplinary SyStaR research team will investigate ageing-related changes in stem cell compartments and regen-

Figure 3: Scientific concept of the SyStaR Consortium

The analysis of signalling pathways and their ageing-related changes involves integrating molecular biological experiments and central systems biology modelling. In an iterative process, the models are refined by repeated experimental testing. The modelling makes it possible to identify key components that may be relevant for therapeutic approaches (Diagram: Hans A. Kestler and Lenhard Rudolph).

erative tissues of model organisms (the Drosophila fruit fly and the mouse), in genetic mouse models of ageing and in humans. The research groups in SyStaR combine expertise in systems biology, ageing research, model organisms used, stem cells, signalling pathways, and geriatric medicine. Over the five-year period of research, the SyStaR Consortium will identify functionally relevant signalling pathways that play a crucial role in the decline in stem cell function and regenerability in the context of human ageing. SyStaR will endeavor to discover whether the identified signalling pathways are suitable as therapeutic targets for the treatment and prevention of regenerative dysfunction and impaired organ function in the elderly. The functional analysis and the validation of the generated models will be conducted in model organisms and in genetic mouse models. These experiments will set in motion an iterative process of model optimization and repeated experimental evaluation. This approach could speed up the transition from mapping the signalling pathways to identifying a suitable drug for a particular condition.

The research project in brief:

The SyStaR research core at Ulm University is being funded by the GerontoSys Initiative of the German Federal Ministry of Education and Research. The objective of SyStaR is to improve our understanding of age-related changes in signalling pathways that impair the self-renewal and function of adult stem cells or the regenerative capacity of differentiated cells.

Participating partners

Mathematical modelling

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- Bioinformatics and Systems Biology Research Group, Institute of Neural Information Processing, Ulm University, PD Dr. H. Kestler
- Institute of Number Theory and Probability Theory, Ulm University, Prof. U. Stadtmüller

Molecular biology research

- Dept. of Dermatology and Allergology, Ulm University Hospital, Prof. H. Geiger
- Institute of Molecular Genetics and Cell Biology, Ulm University, Prof. N. Johnsson
- Institute of Biochemistry and Molecular Biology, Ulm University, Prof. M. Kühl, PD Dr. P. Pandur
- Dept. of Internal Medicine I – Gastroenterology, Ulm University Hospital, Prof. F. Oswald
- Leibniz Institute for Age Research and Max Planck Research Unit for Stem Cell Aging, Ulm University, Prof. K. L. Rudolph
- Institute of Physiological Chemistry, Ulm University, Prof. T. Wirth

Clinical research

- Dept. of Internal Medicine I – Gastroenterology, Ulm University Hospital, Prof. T. Seufferlein, Dr. A. Kleger
- Dept. of Neurology, Ulm University, Prof. A. Ludolph
- Bethesda Hospital, Ulm, Prof. T. Nikolaus, Dr. M. Denkinger
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ISBE – infrastructure for systems biology in europe

by Jutta Steinkötter, Thomas Höfer and James Sharpe

ISBE – Infrastructure for Systems Biology in Europe is a project of the European Strategy Forum for Research Infrastructures (ESFRI), and plans to have a big impact on life scientists across Europe. The ISBE consortium addresses the necessity to link existing infrastructures and establish new ones in order to allow clinicians, biologists, chemists, physicists and computer scientists to collaborate efficiently on a European level in tackling complex biomolecular challenges. The current EU's 'preparatory phase' financing for ISBE establishes a framework for strategic coordination within the scientific community, while the subsequent Europe-wide implementation of infrastructures will involve the engagement of national funding agencies. The systems biology community at large will be informed about and involved in recent developments via an informative session and launch of the ISBE's community web site during the ICSB in Copenhagen in August 2013. Watch out for specific announcements during the conference or @ www.icsb2013.dk!

ESFRI – The European Strategy Forum for Research Infrastructures was established in 2002 with the aim of fostering the joint usage of infrastructures to facilitate international competitiveness in science and technology development in the fields of energy, the life sciences, physics and astronomy, social sciences as well as that of information technology. An intensive advice and consultation process resulted in three calls for 'ESFRI-roadmap' actions and developed into 48 future-oriented thematic initiatives.

Within the life sciences, topics including bioinformatics, structural biology, biobanks, imaging, marine biology along with other high-tech initiatives are supported by the EU for the initial three-year preparatory phase or have already progressed to the subsequent stage of coordinated funding from EU member states for their implementation. Implementation comprises not only the establishment and expansion of infrastructure sites and technologies but also contractual and legal agreements between the partners, sites, users and the participating member states. Both activities are significant challenges! As a success story, the German coordinated initiative EU-Openscreen has already achieved strategic success and is already part of the BMBF's Funding Roadmap and can therefore expect to profit from national funding instruments. It is the explicit goal of the ESFRI strategy to facilitate the implementation of research infrastructures through national funding agencies by establishing the necessary coordination and legal frameworks in the preparatory phase. A complete list of biomedical projects, their status and funding can be found in the current report of the TWG BMS - Thematic Working Group Biological and Medical Sciences (see links below for further info).

In 2011, the European Strategy Forum decided to include systems biology in the list of infrastructure areas and called for proposals. The consortium of ISBE, 23 partners from 11 member states, coordinated by Prof. Richard Kitney from Imperial College London received three years financing, starting in 2012. ISBE now works on the development of infrastructure concepts for systems biology in Europe.

Prior to the ESFRI grant proposal the partners were already active in pan-European measures such as ERA-NET (e.g. ERA-NET ERASysBio) and related initiatives. German consortium members are the Max Delbrück Center for Molecular Medicine (MDC Berlin-Buch), Heidelberg University, the German Cancer Research Center (DKFZ) and the Heidelberg Institute for Theoretical Studies. Germany supports systems biology through large-scale project funding and investment in the establishment of a vibrant research landscape. Now, these sites and expertise can be networked Europe-wide.

Systems biology in Europe would strongly benefit from technology and science oriented infrastructures. The potential for systems-wide functional analysis and modelling of genomic, transcriptomic, proteomic, and metabalomic data is enormous but not yet fully exploited. This is equally so for basic research as well as for medical and translation focused approaches. However, it is becoming increasingly important to address the complex scientific questions of medicine and biotechnology within larger research consortia that make available multiple complementary technologies far exceeding the capabilities of individual groups. ISBE has the mission to establish a strategic concept for an optimal infrastructure, networking, technologies, quality standards and educational concept in order to support systems biology Europe-wide. The components of this distributed infrastructure would be facilities that hold or create relevant technologies, data and databanks as well as expertise in experimental procedures, algorithms and the capacity to analyse or model. They are defined by accessibility of technology applications and data analysis as well as complementarity and interoperability. They will be compatible through such joint facilities and amenable to the scientific user for systems-wide scientific approaches. The combination of different disciplines and technologies makes the network valuable for the user and helpful for the advances in systems biology.

The intensive participation of the scientific community is important to this strategic process - an objective of the consortium and an important political and strategic success factor. In this respect, ISBE will inform and involve future users as well as the broader range of stakeholders through its website and at specific meetings. For example, an open and public event at the International Conference on Systems Biology (ICSB), taking place on August 30 – September 3, 2013 in Copenhagen, is being planned: The ISBE consortium will introduce ISBE's concept and launch the ISBE community website which will facilitate an Europe-wide expert exchange for the design of the infrastructure. Europe-wide surveys will bring together and assess the needs and essential aspects that scientists and institutions consider of relevance for the benefit of systems biology research in Europe.

It is clear that through interdisciplinary technology and data driven infrastructures for systems biology, there will be many points of contact as well as areas of interfacing with existing activities in the natural sciences (please read also "European Systems Biology – A Joint Effort" on page 59). Already active national and European initiatives, research consortia and coordination activities hold, as do the ESFRI initiatives themselves, considerable synergy potential which will also support ISBE goals. ISBE will exchange and organize joint workshops focusing on the overlaps and synergies, which will be beneficial for any of these initiatives.

Further information:

ISBE: http://www.isbe.eu

ESFRI: http://ec.europa.eu/research/infrastructures

ESFRI BMS Report 2010: http://ec.europa.eu/research/infrastructures/pdf/bms_report_en.pdf

ICSB: http://www.icsb2013.dk/

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european systems biology – a joint effort

by Babette Regierer and Susanne Hollmann

Researchers and coordinators of various EU life science initiatives as well as COST Actions participated in a first community building meeting for bioinformatics and systems biology in Amsterdam 2012. The meeting initiated by the Coordination Action "ALLBIO Broadening the Bioinformatics Infrastructure to Unicellular, Animal and Plant Science" discussed current and future challenges in bioinformatics and systems biology identifying interfaces and missing links between these initiatives. Significant interfaces are the management and integration of data, development of standards and training. Systems biology, where the integration of data, methods and knowledge is of crucial importance to elucidate the complexity of cells, organs or organisms, is a huge challenge that cannot be addressed by a single entity or country, but needs a joint effort of many initiatives, institutions and countries. To support systems biology across Europe, ISBE, the Infrastructure for Systems Biology in Europe, was established as a new distributed, but closely interconnected infrastructure (please read also “ISBE” on page 57ff). This will facilitate the synergistic application of a wide range of research techniques and approaches to problems of medical and biotechnological importance for the European bioeconomy. ISBE will also gather and facilitate efficient interaction with the relevant substantial technology development and knowledge generation efforts across whole Europe and beyond.

The interfaces between ISBE and other initiatives that started independently will leverage the potential of systems biology. To use the opportunities that emerge from the interaction is a huge added value. A selection of various initiatives, particularly in the areas of data standardization and training connecting to ISBE is introduced here. Other initiatives are therefore invited to get in contact to use potential synergies and collaboration.

AllBio

Broadening the Bioinformatics Infrastructure to Unicellular, Animal and Plant Science

www.allbioinformatics.eu

Over the years a large number of novel analytic and predictive computer programs, bioinformatics tools and web services were created. As the majority of these developments focus on human, one central aim of AllBio is to transfer the knowledge of existing tools to the other various life science areas, i.e. plants, microbes, animals, and to identify still unsolved bioinformatics challenges (see also page 74 for AllBio Workshop).

CASyM

Coordinating Action Systems Medicine

www.casym.eu

CASyM is a strategic platform to develop a roadmap for the implementation of systems medicine across Europe. CASyM combines 22 partners from research, health care, companies, funding bodies, research clusters and project management agencies. CASyM assesses the basis for an European systems medicine paradigm and assists the medical community to create the foundation for a personalized, predictive, preventive and participatory (4P) medicine (read more on page 80).

COMBINE

COmputational Modelling in Biology Network

www.combine.org

COMBINE coordinates the development of various community standards and formats for computational modelling, initially in Systems Biology and related fields. The aim is to create a common platform for allied standardization efforts – including all aspects of modelling in biology – and connect the different communities. The core set of COMBINE standards currently comprises eg. BioPAX (Biological Pathway Exchange format), or the SB Markup Language (SBML). Building on the experience of mature projects, which already have stable specifications, software support, user-base and community governance, COMBINE will help to foster fledging efforts aimed at filling gaps and address new needs.

ERASys*APP*

ERA-Net for Systems Biology Applications

www.erasysapp.eu

ERASys*APP* is a novel ERA-Net of 13 European countries to coordinate and enhance research opportunities in applied systems biology. The first call in November 2013 is open for any topic and organism in systems biology.

EraSynBio

ERA-NET for the Development and Coordination of Synthetic Biology in the European Research Area

www.erasynbio.eu

ERASynBio is an ERA-NET joining 14 countries aiming at coordinating national and regional funding programmes to foster developments in the field of synthetic biology.

SysMO-DB

For finding, sharing and exchanging Data, Models and Processes in Systems Biology.

www.sysmo-db.org

SysMO-DB is a web-based platform with tools for finding, sharing and exchanging data, models and processes in systems biology to support the SysMO consortium (Systems Biology for Micro-Organisms). The main objectives of SysMO-DB are to facilitate the web-based exchange of data and provide an integrated platform for the dissemination of the results to the scientific community.

GOBLET

Global Organisation for Bioinformatics Learning, Education & Training

www.mygoblet.org

High-throughput biology, especially NGS, generates vast quantities of complex data that require detailed and accurate analysis. GOBLET aims to address these issues by providing a coherent platform to disseminate learning programmes across the complete bioinformatics landscape. To date, 24 international organisations joined GOBLET with the mission to provide a global, sustainable support structure for bioinformatics trainers and trainees, and facilitate capacity development in bioinformatics in all countries.

ELIXIR

European life science infrastructure for biological information

www.elixir-europe.org

ELIXIR unites Europe's leading life science organisations in managing and safeguarding the massive amounts of life science data. It is a pan-European research infrastructure for biological information. It follows a hub-and-nodes model, with a single hub located at EMBL-EBI (UK) and a growing number of nodes throughout Europe. The goal of this distributed structure is to ensure data safety and accessibility. ELIXIR coordinates also **BioMedBridges** bringing together ESFRI Research Infrastructures in the biomedical sciences to create a shared e-infrastructure with interoperability between data and services in the biological, medical, translational and clinical domains.

SystemsX.ch

The Swiss Initiative in Systems Biology

www.systemsx.ch

SystemsX.ch comprises 12 partner institutions and supports over 1.000 scientists and 300 research groups working on more than 100 projects on systems biology in Switzerland. SystemsX.ch includes the promotion and training of future systems biologists with Interdisciplinary PhD Projects (IPhD) and Transition Postdoc Fellowships (TPdF). The transfer projects encourage cooperation between universities and the private sector.

The joint effort between the different projects, initiatives and infrastructures will have a huge impact on European industry, business and society through the systematic study of complex biological processes and incorporating expertise derived from other science fields. This will lead to new and important applications; e.g. in health, agriculture, food, energy and other areas related to the bio-based economy of Europe. The systems biology approaches and the various initiatives enabled by infrastructures like ISBE will provide new insights and assist the development of tools for the design of new applications. The field of systems biology will be transformed into an integrated, pan-European activity by linking all expertise needed. Biological sciences will be more productive and cost-effective, which is important for European science, society, industry and business in nearly all areas of modern life.

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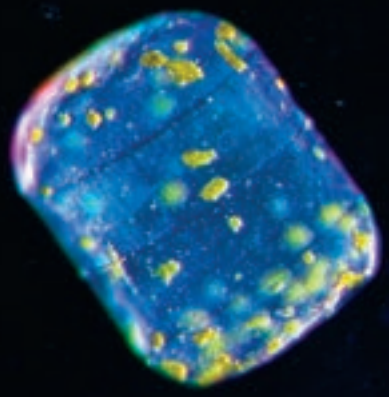
TARA OCEANS

A world wide marine plankton sampling project with systems analysis in mind



Diatom *Coscinodiscus sp.* **(Diatoma)** (Photo: C. Sardet CNRS / Tara Oceans).

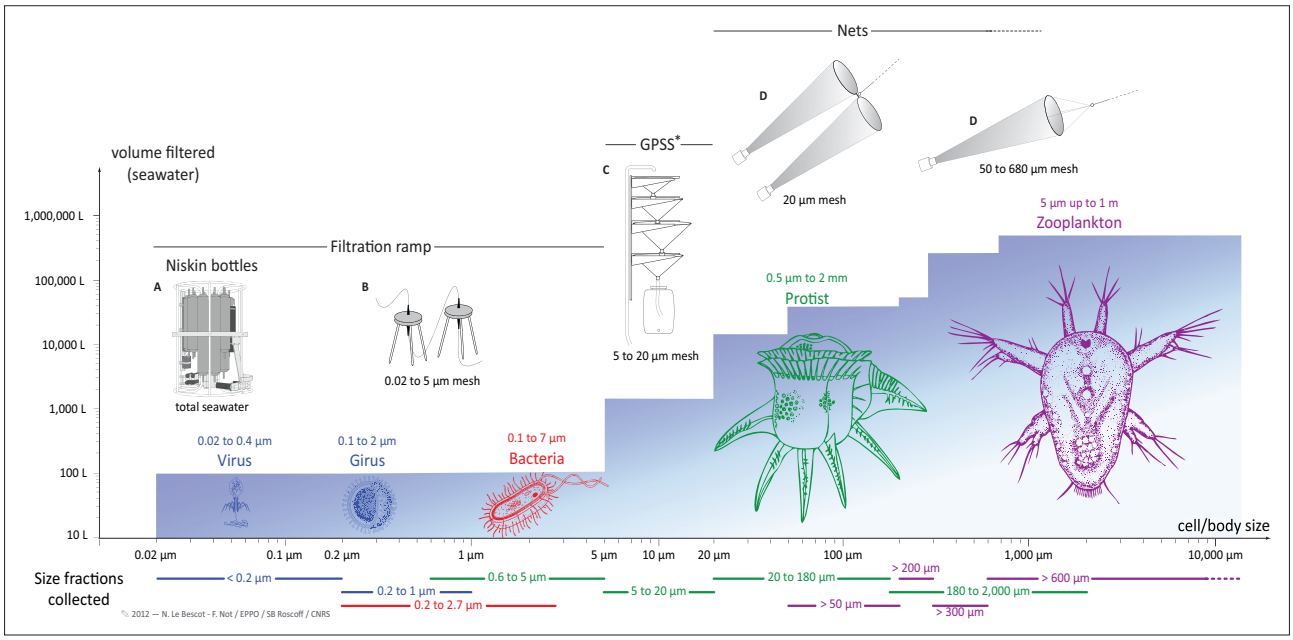
by Eric Karsenti

The earth system and the ocean

When you take a swim and swallow a mouthful of sea water, you take up a large number of viruses, bacteria, protists and small metazoans. There are 1 to 10.000 metazoans, 1 to 100 million protists, 1-10 billion bacteria, 10 to 100 billion viruses per liter of seawater. Interestingly, most of the time this does not make you sick. Indeed, it is a fact well known from sailors that the sea is a healthy and invigorating environment. This is telling: the organisms that live in the oceans do not really interfere with us. They have evolved as a living system in oceans for billion years and although they are our distant ancestors, we evolved over the past 100 million years on land. Our biology became foreign to them and yet without their first emergence, evolution and activity, our development would not have been possible: they made our blue oxygenated planet (Falkowski, 2006).

Life started in the oceans some 4 billion years ago in an atmosphere composed of more than 90 % CO_2 and no oxygen. About 3 billion years ago, the oxygen rose to a few percent in the atmosphere, owing to the appearance of the first photosynthetic bacteria. The situation remained like that for another 1-2 billion years until the first photosynthetic protists emerged from a symbiotic event between a eukaryotic cell and some kind of photosynthetic "bacterium" that we call today chloroplasts (Falkowski, 2004). These photosynthetic

Figure 1: Strategy of sample study



Shown are the methods by which the organisms are sorted by size and frequency. The blue background indicates the filtered volume that is required to obtain a sufficient number of organisms for analysis (Grafics: N. Le Bescot and F. Not; Karsenti *et al.* 2011).

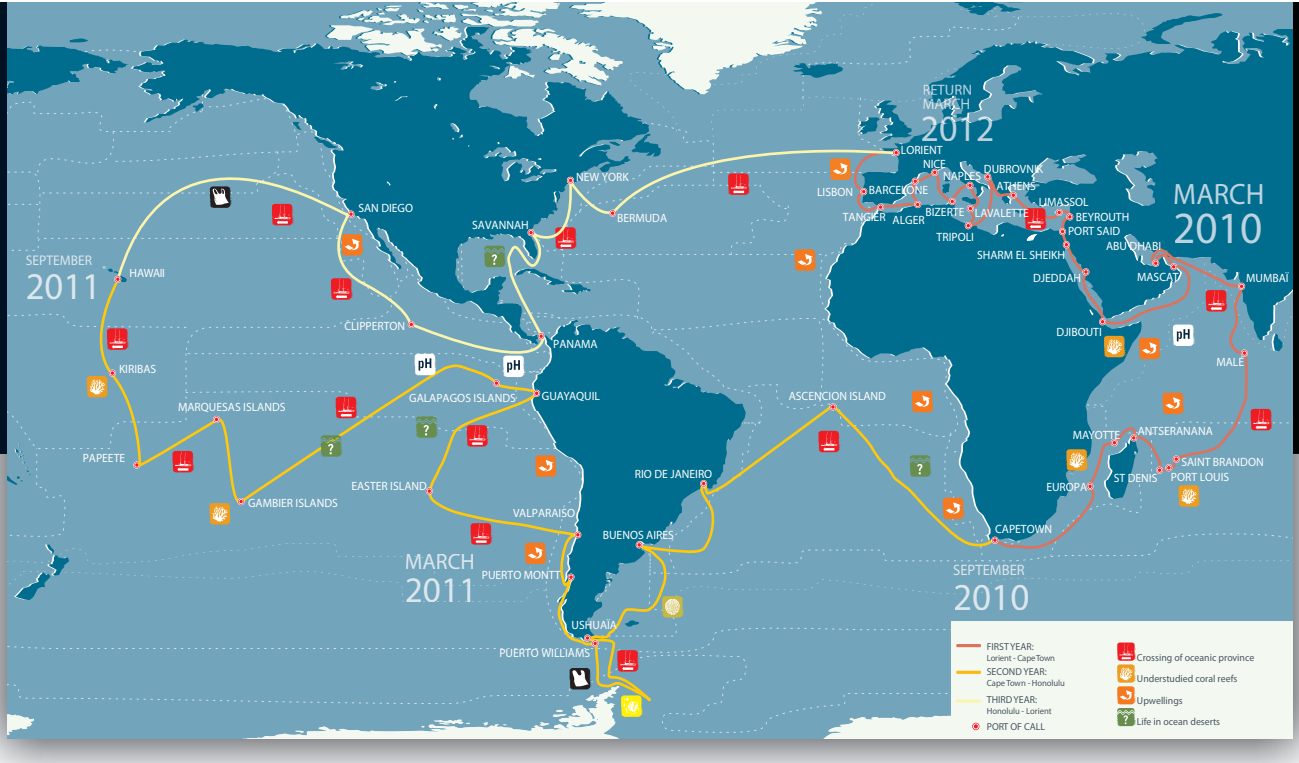


Figure 2: Itinerary of the expedition TARA OCEANS (Grafics: N. Le Bescot and F. Not; Karsenti *et al.* 2011; Be-poles.com/Tara Expéditions).

cells became very complex, acquiring silica or carbonate skeletons. These protists increased the production of oxygen, but most importantly, because of their glass or calcareous skeletons, they sunk to the sea bed, removing even more CO_2 from the atmosphere by capturing it in sediments, rocks and petrol. The further diversification of life into multi-cellular forms increased again the sedimentation of organic matter to the sea bed and removal of CO_2 from the atmosphere, leading to the present day atmosphere with its high Oxygen concentration (20%). This is what allowed the evolution of mammals and the huge diversification of sea and land life that we know of today.

What I just described is, very simply put, the earth system. Life and geology have interfered with each other and are still doing so to shape our living planet over eons. The notions of systems, shape and function are intimately related. Systems biology is exactly about this issue: people working in this field are not only trying to identify the components of living systems but rather to understand how interaction networks are structured in order to generate functional entities (Karsenti, 2012). Shapes emerge from the interaction between various agents and functions emerge from specific shapes or patterns. At the same time, functions affect the interactions between agents, leading to shape formation through a circular logic (Karsenti, 2008). The evolution of the earth system I described is built on this logic: organisms evolved in a certain environment that they changed by their activity. I mentioned mostly the CO_2-oxygen system, but life has also created coral reefs that support life diversification and huge mountains of dead calcified organisms that shaped lands and land ecosystems. In turn, the new forms of life that emerge also change the planet. Now human beings have proliferated dramatically and begun to interfere heavily with the whole system. In a hundred years, we have released into the atmosphere large amounts of carbon dioxide that had been captured in fossil fuels over million of years. This is threatening to interfere with both the climate and the ecosystems as a whole rapidly and in unknown ways. Beyond taking political actions on a global scale, we need to develop quantitative dynamic models to understand and possibly predict the evolution of the earth. One needs to use mathematics and computer simulations because the system is built of stochastic interactions and feedback loops that make it non linear.

Apart from the theory, we still do not have a satisfactory description of the earth system. Even the theory of the evolution of life is very primitive. Moreover, we have very little data to feed models, especially about the plankton organisms that started it all and who are still the key players of the system in general. The goal of TARA OCEANS is to initiate an approach for gathering the right type of data and to promote an integrated interdisciplinary approach to the understanding of ecology and evolution of marine life.

Zooplankton of the genus *Platynereis* of the Gambier Islands in the South Pacific (Photo: E. Roettinger/Kahikai/Tara Oceans).

The genesis of the TARA OCEANS project

The TARA OCEANS project is an initiative that started through my incentive. I first discussed the idea with friends in the Marine Biology station of Villefranche-sur-Mer in the south of France. Christian Sardet was an old friend, a cell and developmental biologist, who had a long lasting love for the beauty of Plankton organisms. He and the oceanographer Gaby Gorsky strongly contributed to the development of automated imaging machines and sophisticated CTDs (*Conductivity, Temperature, Depth*; probe for deep sea studies equipped with sensors for conductivity, temperature and water pressure) and rosettes to collect very high quality oceanographic data. We planned to collect samples and data to better describe the structure of global plankton ecosystems. In parallel, with colleagues at EMBL (Peer Bork, Jeroen Raes, Detlev Arendt and Rainer Pepperkok), I discussed the possibility of doing massive sequencing and bioinformatics coupled to imaging to get some kind of association between genes and plankton shapes for the first time.

After having met with the owners of the 110 foot schooner TARA (the agnès.b family) which had been used for scientific expeditions, I looked for colleagues, which are specialists of the various kingdoms of life. Through discussions with Chris Bowler and Christian Sardet, I met Colomban de Vargas who got Jean Weissenbach and the *Genoscope* (French National Sequencing Centre in Evry close to Paris) on board of the project to do the sequencing. We ended up with a consortium that brings together a strong interdisciplinary team (Karsenti, 2011). This team engineered the sampling plan carried through the entire voyage and is now analyzing the samples. I think that this is a unique experience and should prove extremely powerful for the analysis of such a complex system as the world pelagic plankton ecosystem.

It took a relatively short time to prepare this enormous project. Once the team was put together, we started off with regular meetings every three months to prepare the expedition. This has continued during the expedition and will con-

Eric Karsenti and Etienne Bourgois, Co-directors of TARA OCEANS expedition, explore with their team on the schooner TARA the ecosystem of the oceans (Photo: F. Aurat/Tara Expéditions).

Annelid *Tomopteris sp.* (Annelida) (Photo: C. Sardet CNRS/Tara Oceans).

tinue during the analysis of the results and ensures a highly coherent approach to the work and constant improvements through regular confrontations of ideas. Given the various backgrounds of the team members, this also ensures a self-teaching process. It was not and still is not easy to get hard core oceanographers to understand the often obscure language of molecular biologists and bioinformaticians as it is not always easy to get the molecular biologists to understand the importance of water masses, gyres and complex transport processes. As such, this is a wonderful example of the emergence of an interdisciplinary project leading to cross disciplinary fertilization encapsulating exploration, analysis and model building phases. In other words: a perfect example of systems biology at the global level.

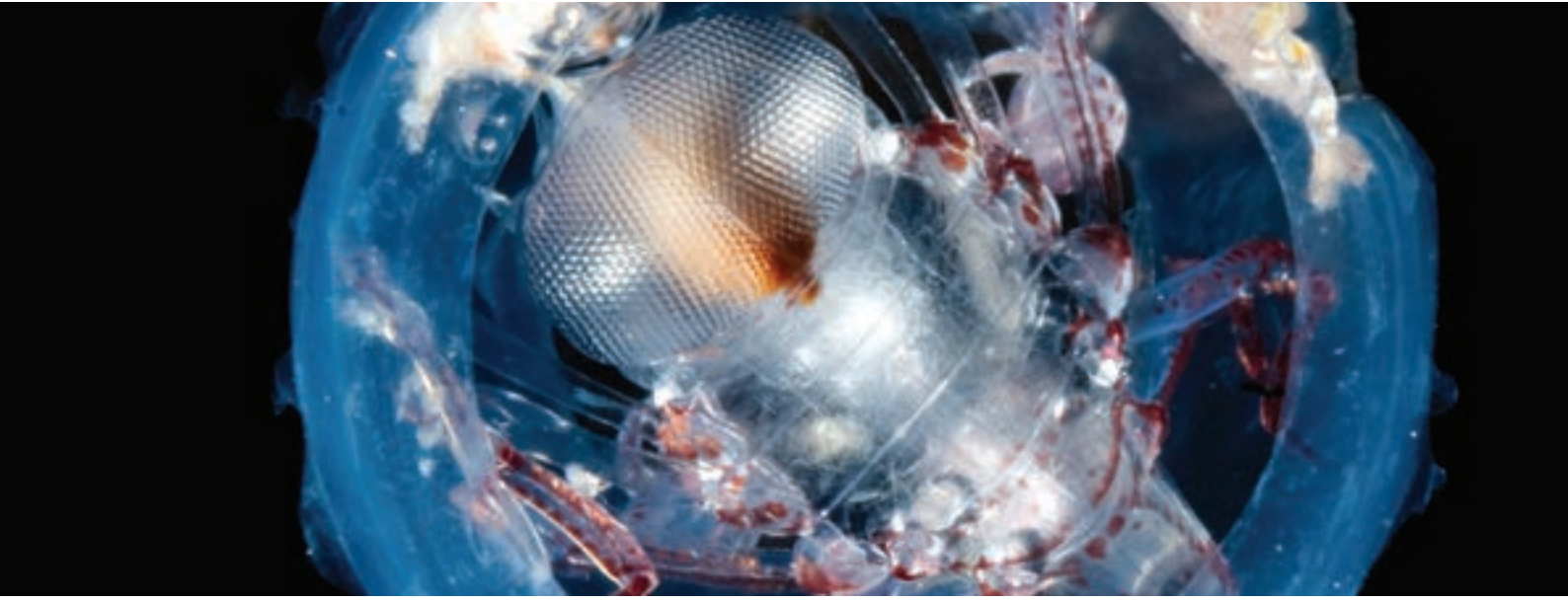
TARA OCEANS and planetary systems biology

The plankton system is hard to study because of its large temporal and spatial dimension. Pelagic plankton organisms have turn over times in a range of hours to days, are transported by the global ocean circulation over months to years and they evolved over billions of years. Oceanographers and marine biologists know very well also that the composition in organisms varies according to day and night and with seasons. To be fully exhaustive in the characterization of pelagic ecosystems, one would therefore like to get all organisms present in a water column in as many sampling stations as possible around the world, during day and night and repeat this at regular times over the year. It is necessary to count organisms according to kingdoms (viruses, bacteria, protists and zooplankton) because this forms a complex interaction network. But they also have to be analyzed at the species level to get a feeling of the evolution of the system!

Physicists have learned both to treat enormous sets of data and to determine what are the essential parameters to record in order to build initial models that, even if not perfect, will give a hint at what to do next to get closer to an understanding of a complex system.

We have followed this wisdom and decided on four important strategies:

1. To sample and separate organisms by size classes from a few microns to millimeters by using various nets and filtration devices in a way that would allow their identification and quantification using high throughput sequencing methods as well as automatic imaging methods (Fig. 1).
2. To sample at three depths: Surface, Deep Chlorophyll maximum (DCM between 20 and 60 m deep where most of the photosynthetic organisms live) and in the Mesopelagic layer (300-500 m deep) just below the photic layer. This defines a typical TARA OCEANS station.
3. To acquire essential environmental data (temperature, salinity, pH, nitrogen, chlorophyll, particulate matter and many others) at each station and characterize the water column down to 1000 m by several CTD casts.
4. Not to do standard transects across large ocean basins but rather target “typical water masses” in each basin, as well as water masses migrating between oceanic basins, in order to get the beginning of an information about the role of global oceanic transport in the organization of ecosystems, their dynamics and evolution, even in the absence of time series.



Amphipod *Pronima sp.* (Amphipoda) (Photo: M. Ormestad/Kahikai/Tara Oceans).

This resulted in 153 stations in most oceans, except the North pole, leading to about 28.000 biological samples correlated with precise environmental parameters and water masses, the history and future of which can be traced using satellite data (Fig. 2).

On the analysis side of it, ecosystems are quantified using imaging devices that allow the quantification of organisms from viruses to zooplankton (Karsenti, 2011). We use metagenomics, metatranscriptomics as well as ribosomal genes to characterize the diversity index, the genomic complexity as well as the metabolic activities present in each water mass sampled. Samples are also kept at each station to carry out single organism genome sequencing in order to have reference genomes from the very ecosystems on which metagenomic studies are carried out.

The types of models that will be fed by this sampling approach have been described (Karsenti, 2011). This should, for the first time, allow to put hard numbers on the diversity index of pelagic ecosystems, and on correlations between ecosystem composition and physico-chemical parameters. It should help to redefine the world "biogeography" of plankton ecosystems and determine whether seasonality can be reduced or not to key physico-chemical parameters, potentially allowing to predict ecosystem composition changes. Moreover, this should also provide data about the physiology and metabolic activities of water masses and finally inform us on the ongoing evolution of ecosystems in the oceans world wide. All of this is key to advance towards a true scientific and unbiased knowledge of the potential impact of important and rapid temperature changes on ecosystem compositions. Obviously, the TARA OCEANS expedition will not be sufficient, but we already have made a huge progress.

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networks of molecules

Interview with Thomas Höfer
by Claudia Eberhard-Metzger

Thomas Höfer studied biophysics in Berlin and received his doctorate in mathematics from the University of Oxford. But the really interesting research themes, he subsequently concluded, are currently to be found in biology. Working at the German Rheumatism Research Center in Berlin, Höfer first investigated the immune system's memory and in doing so helped to find out how a pathologically changed immunological memory causes repeated inflammatory rheumatic flare-ups. For the past five years, Höfer has been doing research at the German Cancer Research Center (DKFZ) in Heidelberg and at the University of Heidelberg's BioQuant Center.

Systembiologie.de: *Professor Höfer, your field is theoretical systems biology. That sounds dry as dust – what's it all about?*

Prof. Dr. Thomas Höfer: The fundamental processes in the life of a cell are controlled by complex networks of interacting molecules. This is what the research in molecular biology and genetics over the past few decades tells us. To analyze these networks, we apply mathematical methods hand in hand with quantitative experimental techniques. What interests us most in our efforts to understand biological functions are the dynamics of these networks. Biology is undergoing a transformation here. Quantitative work involving mathematical concepts has been a niche for a long time, whereas these days, especially for young scientists, it is becoming mainstream – and an amazingly exciting field to work in!

Do you have any examples for us?

The immune system and regulation of the immune system is a good example of a highly complex, dynamic system. We want to know how certain white blood cells build up the immunological memory. What we still lack are fundamental insights that would enable us to develop targeted vaccinations to activate the immune system against cancer and to treat autoimmune diseases more effectively.

And what exactly can mathematics contribute to a better understanding of such dynamic biological processes?

Let me take an example from physics to explain what I mean. You could of course ask what is the point of describing the law of gravity in mathematical terms, since we all know that what goes up must come down. But had the law of gravity not been formulated as an equation, we would not be able to send satellites into space now – to name just one example. Galilei and Newton probably had no inkling of that back then. It seems to me that some of the issues currently of concern in biology should be approached in the same way.

And what makes you think that?

Some experiments in biology can no longer be interpreted without mathematical models. One long-standing question in immunology is whether memory cells come into being right at the start of an immune response to a pathogen and hence are the immune response's stem cells, as it were, or whether they arise later, as a product of so-called "effector cells," in which case they are a fully differentiated cell type. This is very important for vaccine research. Despite numerous experiments, people still argue quite fiercely about this question, mainly because we presently cannot observe the differentiation of an individual immune cell directly in an intact organism. Using mathematical modeling, however, we found that a unique experimental technique developed by our cooperation partner, Dirk Busch of the Technical University of Munich, yields a "footprint" of the immune cells' differentiation path that then can be reconstructed in the computer. In turn our model made predictions which our colleagues in Munich were able to test empirically. The result is unambiguous: Memory cells are the first type of cell to arise at the start of the immune response; none of the alternative models proposed came even close to describing the data.

Thomas Höfer leads the department of Theoretical Systems Biology at DKFZ Heidelberg (Picture: T. Höfer).

So what comes first, the mathematical model or the biological question?

Always the biological question. Only when the question has been formulated can we ask: What kind of mathematical model will help us answering it?

And how do you go about developing it? Can you explain it on the basis of a recent research project, perhaps?

Together with our experimental partners at the DKFZ, we have begun investigating how cells respond to DNA damage. We already know a lot about the molecules that repair the damage to genetic material and the molecules that in the event of serious genetic defects trigger programmed cell death, so-called apoptosis. But how does a cell know whether repair is worthwhile or whether suicide would be the better option to protect the organism against the consequences of serious genetic mutations such as cancer? Basically, this is a quantitative question. To answer it, we need to understand how many different molecules interact dynamically; the traditional idea that there are just a few "rate-limited" steps in such systems turns out to be fiction rather than fact. In our experience, many different steps exert small or moderate influences to control and tune the behavior of the system. We discuss these matters intensely with our colleagues in the laboratory and then translate our knowledge and our hypotheses into mathematical models. One important question now is this: Can the model describe the known data or are there significant gaps in our knowledge? Typically the latter is the case and that's when we go back to the bench, for experiments to measure just that. This continuous iteration between theory and experiment leads us to questions – and answers – which would not exist without the mathematical model. It is one of systems biology's new research strategies.

How might this new way of gaining scientific knowledge be applied in practice?

The example just mentioned is specifically aimed at finding new therapeutic approaches to neuroblastoma, a tumor that occurs in children. In molecular terms, neuroblastoma is very well characterized; medically, however, we need new approaches to target therapy-resistant cells – an overriding problem in cancer research. It's a complicated problem, but I'm confident that we will be able to make a genuine contribution here.

What is the connection between rheumatism research, the field you worked in previously, and cancer research?

On the one hand, both have to do with fundamental questions concerning about dynamic principles underlying cell behavior. Whether or not a cell divides is regulated by complex molecular switches that integrate information obtained both from external signals and from the inner condition of the cell. We have already identified just such a bistable switch mechanism in T lymphocytes, for example, a cell type of the immune system. The experience gained is now proving very useful to us in our study of the cell division of cancer cells. On the other hand, the mathematical tools used in the two fields are also very similar. Mathematics is a comparatively new tool in biology and we are using it to search for answers to key questions about life and using our insights to find new ways of treating complex diseases.

Thomas Höfer talked to Claudia Eberhard-Metzger.

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how much systems biology does the *virtual liver* need?

by Adriano M. Henney

Before attempting to answer the question, we must first clarify what we mean by systems biology. That may seem odd, but it is true to say that, even after so many years of practice, there remains confusion in the minds of many over what the term covers. That's clearly not the case for those of us that are studying the dynamics of biological complexity through the application of modelling and simulation to generate testable hypotheses. We all understand that the technological advances of the post-genome era have enabled the rapid acquisition of information at varying levels of complexity. In turn, this has led us to be awash with data and the challenge of how to interpret it all in the context of function, phenotype and physiology, in order to have an impact on modern therapeutics and medical practice.

Eighteenth Century biology and medicine faced a similar challenge following the explosion of information emerging from the invention of the microscope by Hooke and van Leeuwenhoek. It took Linnaeus to devise a classification system based on morphology to organise the data, but this was still descriptive and couldn't establish a link to disease. The explosion of data today presents us with a parallel challenge, albeit at a more complex level. As Sydney Brenner said in his Nobel Prize interview:

"We are drowning in a sea of data and yet we are thirsty [for knowledge].....There is now a crisis developing in biology that completely unstructured information does not enhance understanding, and what people want to do is to understand, which means you have to have a theoretical framework in which to embed this..."

And Dennis Noble offers a potential solution that will not be news to the majority of the readers of this journal:

"It is about putting together rather than taking apart, integration rather than reduction..... Systems biology is set to revolutionise the fundamental principles of biology, including the relations between genotypes and phenotypes.... to succeed in this, systems biology will need to develop the theoretical framework required to deal with multilevel interactions"

This is how we interpret things in the *Virtual Liver Network*, which we regard as a multidisciplinary, multilevel challenge for systems biology, where the multidisciplinary teams working together cover traditional experimental laboratory skills in cell and molecular biology, the theoretical sciences represented by mathematics and physics, as well as engineering and most importantly, medicine.

Adriano M. Henney (Photo: Wort & Bild Verlag / Jürgen Lösel)

Systems biology can accelerate the transfer from academic research to use on patients and can cut costs in the development of medications. That's why it is a key technology and a driving force of innovation for individualized medicine of the future.

Press Release 116/2010 of the Germany Federal Ministry of Education.

This statement was made at the launch of this ambitious programme, funded by the German Federal Ministry of Education and Research (BMBF). This flagship research initiative, arguably the only one of its kind in the world financed at this level by a single national organisation, that focuses effort on a single organ across multiple scales of complexity is now half way through its five-year funding. The network's goal is to create a computer model of the liver that is a representation of the complete organ, incorporating all of its diverse and essential functions.

A biochemical factory in the body

The liver is unique: as the central metabolic organ of vertebrates it synthesizes, converts, and breaks down more than 10,000 substances every day, helping the body to digest food, controlling iron uptake and synthesizing vital proteins such as coagulation factors. Liver metabolism is a critical element in the elimination of toxic substances, including drugs and alcohol. This is a function that is of fundamental importance for drug development as it is central to toxicity. The exploration of the liver and its functions, and the ambition to generate a multiscale model that can simulate the dynamics of its function is therefore of the greatest relevance both to medicine and the pharmaceutical industry.

A multiscale systems approach to understand physiology

In order to get an overall picture both of the liver as a whole and of the diverse and dynamic processes in the organ, the network's researchers are adopting systems biological approaches, but are operating across a wide range of scales of temporal and spatial organisation. The exploration of biological processes at the systems level seeks to create a holistic picture of dynamic life processes at all levels – from the genome to the proteome and up to the complete cell and even an entire organism. In order to achieve this goal, quantitative methods from the field of molecular and cellular biology are closely integrated with techniques and tools from the areas of mathematics, physics, engineering and computer sciences.

From the cell to the whole organ

Over a number of years, a significant amount of effort has been devoted to modelling cellular networks, looking at signalling cascades and metabolic pathways in particular. The challenge now is to move out of the cell to consider higher levels of organisation in tissues and organs. This reflects the focus of the activities within the *Virtual Liver Network*, which in many ways is providing a blueprint for the integration of "bottom-up" mechanistic modelling approaches, and those adopting a more physiological/physics-driven, "top-down" approach exemplified by the Virtual Heart, and as such represents a logical next step in the evolution of systems approaches: the ability to generate simulations across scales of time, space and organisation.

Our objective is to generate experimentally testable predictions that are relevant to the physiology of the liver, as well as the function of the whole organism. Our approach is unique in that the traditional systems approaches are complemented by the inclusion of experimental clinical units with ethical clearance for studies in human volunteers. This integrated effort across biology, physics and medicine will contribute to an improved understanding of the liver as the body's most important metabolic organ and how its normal function is affected in disease. By using validated simulations, these models will greatly benefit efforts to find new therapies, to predict how active substances distribute themselves in the organ, where they attack, and how quickly they are broken down. Thus, medications can be developed in a more targeted, more efficient and cost-effective manner to focus on delivering the optimum dosage to the right patient at the right time. With the growing emphasis on the personalisation of therapies, the role of systems approaches to interpret and understand how network dynamics influence emerging phenotypes, will become even more important. Evidence of the impact coming from studies in the *Virtual Liver Network* will help to build confidence in systems approaches and consolidate its relevance to modern biomedical research as well as, ultimately, medical practice.

A world leader

The *German Virtual Liver Network* is the first project worldwide to aim at building a truly multi-scale computer model of a complete organ – from biomolecular and biochemical processes up to the anatomy of the whole organ, and ultimately the individual. The challenge is immense, but the potential rewards are significant, not only in improving our understanding of the liver, generating novel tools and processes, but as importantly in generating a strong impetus to the entire area of systems biological research by providing evidence of genuine impact on healthcare.

So to answer the initial question, *"how much systems biology does the virtual liver need?"*, the answer is: a considerable amount! Additionally, the traditional moulds are broken to work beyond the confines of the cell and to include higher levels biological and physiological organisation.

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# of mice and models

Geneticists and systems biologists meet at the "Berlin Summer Meeting 2012"

by Alexander Löwer

Rain or sun, summer is the time for experimental and computational biologists to meet at the Berlin Summer Meeting. Initiated in 2008 by Nikolaus Rajewsky, this annual event is organized by the Berlin Institute for Medical Systems Biology. Over the past years, the number of participants increased steadily to reach more than 250 internationally recognized scientists in 2011. Under the topic "RNA, Protein and beyond", they exchanged ideas and discussed the latest developments in systems biology. In 2012, a different format was chosen for the Berlin Summer Meeting: Klaus and Nikolaus Rajewsky invited excellent scientist from all over the world to join researchers from the Max-Delbrueck-Center for a three day retreat to Döllnsee in the beautiful Schorfheide north of Berlin.

The idea behind the retreat was to contrast two of the most promising approaches for understanding the complexity of biology: genetic dissection vs. systems biology. For decades, geneticists devised more and more sophisticated methods to modify the genomes of their favorite model organisms and used the resulting changes in phenotypes to deduce the function of the altered gene. However, this can only be done for one gene at a time, and each perturbation induces complex changes in the very system that is studied. Here, systems biology provides a different approach by looking at a biological

At the Berlin Summer Meeting, participants discussed on systems biology and genetics not only during the numerous lectures, but also in the breaks.

Photo: Lena von Oertzen

The Döllnsee in the Schorfheide north of Berlin provided the ideal environment to discuss opportunities and challenges of interdisciplinary collaborations of biologists, physicians, mathematicians and physicists (Picture: Lena von Oertzen).

system as a whole and considering all the molecular reactions that take place in a cell as part of an interconnected network. To achieve this, systems biology combines high throughput data with theoretical analysis and computational modeling.

Named after one of the founders of modern genetics, the MDC is renowned worldwide for its expertise in molecular genetics. Many of the sixty research groups use this approach to study the causes of human diseases. At the same time, the MDC is a prominent center for systems biology in Europe. The "Berlin Institute for Medical Systems Biology" already hosts eight research groups and three technology platforms and more will join in the near future. Therefore, the MDC is an ideal place to discuss what geneticists and systems biologists can learn from each other and how the strengths of both approaches can be combined to answer the big questions of our time.

This can be best illustrated by a brief example. Micro-RNAs are small pieces of genetic information that do not have the capacity to produce a protein. Nonetheless, they have strong influence on the fate of a cell. Computer algorithms and high-throughput experiments revealed that each micro-RNA has the potential to target thousands of other molecules. Geneticists can now test the impact of the regulation of these predicted target genes. Often they find that only one or two of the predicted targets are relevant for a given effect of a micro-RNA. Hence, the question remains whether the other targets are relevant as well and if yes, in which context? And why are there so many to begin with?

These and other questions where discussed during the intense scientific program that featured talks from international guests such as Fabio Piano (New York University), Neal Copeland (MHR Institute Houston) or Steve Cohen (IMCB Singapore) as well as from MDC group leaders and scientists from across Germany. Soon it became obvious that already today there are no clear boundaries between systems biology and genetics. The broad spectrum of science presented can be condensed into four questions: What are all the molecular parts needed to make a cell work? How do these parts interact in networks to fulfill their function? How do genes and molecular networks control faithful development? And how do perturbations of these networks cause diseases?

A good example for the merging of genetics and systems biology is the research from Fabio Piano's lab. The aim of his group is to fully understand early embryonic development. To this end, they use the nematode *C. elegans* as a paradigm to systematically analyze the temporal and spatial activity and function of every gene. They identified a complex network

Participants of the Berlin Summer Meeting 2012 (Picture: Lena von Oertzen).

that is constantly reconfigured. In this network, proteins form molecular machines and perform complex functions by mutual regulation. Almost every gene contributes, to varying degrees, to the successful development of the whole organism.

Networks are not only important during development, but also play a crucial role in diseases. Neal Copeland's group, for example, used genetic techniques to comprehensively study tumorigenesis. They discovered that hundreds of genes are involved in this process, but all of them participate in only a handful of molecular networks. The order in which this central networks are inactivated during tumorigenesis determines which specific genes are activated or inactivated. These are important results with regard to diagnosis and therapy of various cancers.

At the end of the retreat, scientists agreed that genetics and systems biology will have to move closer together in order to address the complex questions still open in biology and medicine. Key to this is fostering interdisciplinarity by combining new approaches, techniques and points of view from physics, mathematics and engineering with the experience and knowledge of biologists. There are various ways to promote interdisciplinary thinking and collaboration. While personal interactions between scientists will always play an important role, education is central for laying the groundwork for effective communication and collaboration across disciplines. The MDC is in the unique position of combining strong expertise in classical fields of biology, such as mouse genetics and molecular biology, with a strong and growing initiative in systems biology. This creates the opportunity to train scientists with a broad perspective and a diverse set of skills and enables advances in basic and translational research.

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events

ICSB 2013 – 14th International Conference on Systems Biology

August 30 – September 3, 2013, Tivoli Center
Copenhagen, Denmark

The annually upcoming International Conference on Systems Biology (ICSB) is the world's largest and most important conference of the systems biology research community. Founded in 2000 by the ISSB (International Society of Systems Biology), the 14th ICSB will take place this year in Copenhagen on August 30 – September 3, 2013 at the Tivoli Congress Center. Designed by architect Kim Utzon, the building offers some of the very best in Danish design and conference facilities.

Internationally renowned scientists will report on biomedical research, health care, and drug development in the context of systems biology. The program promises excellent presentations, poster sessions, round-table discussions and workshops around the conference.

More information and registration:
www.icsb2013.dk

AllBio-Workshop on Standardization

ICSB, September 3, 2013, Tivoli Center
Copenhagen, Denmark

A central aim of the AllBio consortium is to transfer the knowledge of existing bioinformatics tools and web services among the various life science areas and to identify still unsolved bioinformatics challenges. Previous work in AllBio revealed that among others standardization was one of the major issues of interest for further cross-cutting activities. As systems biology integrates heterologous and complex data in model approaches, the need for standards in data generation and analysis becomes an urgent topic. A workshop in the frame of the ICSB (International Conference on Systems Biology) 2013 in Copenhagen will give an overview about ongoing initiatives on standardization (e.g. COMBINE) and discusses the benefits of standardization and Standard Operating Procedures (SOPs). It also offers fundamental information on standardization processes as a tool for future industrial application.

Read more on: www.icsb2013.dk

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International Symposium on Synthetic Biology – from understanding to application

December 9 – 11, 2013, DKFZ Heidelberg, Germany

The international symposium on "Synthetic Biology – from understanding to application" is hosted by the Helmholtz Initiative on Synthetic Biology and will take place on December 9-11, 2013 at the German Cancer Research Center (DKFZ) in Heidelberg. It covers a diversity of synthetic biology topics ranging from fundamental research to applications in health research and biotechnology. The scientific program with internationally renowned scientists will be complemented by presentations of the iGEM 2013 Awardees as well as by an Art & Science exhibition. Scientific developments as well as ethical concerns and governance issues will be discussed in a public event.

Confirmed speakers are Luke Alphey (University of Oxford, UK), Michelle Chang (University of California, Berkley, USA), John Glass (J. Craig Venter Institute, USA), Jeff Hasty (University of California, San Diego, USA), Petra Schwille (MPI for Biochemistry, Martinsried, Germany), and Eckard Wimmer (Stony Brook University, New York, USA).

The symposium is organized by the Helmholtz Initiative on Synthetic Biology in cooperation with BBAW – Berlin-Brandenburgische Akademie der Wissenschaften, BIOSS – Centre for Biological Signalling Studies (Freiburg), HeiKa – Heidelberg – Karlsruhe Research Partnership, DECHEMA – Society for Chemical Engineering and Biotechnology, and NSB-Upper-Rhine – a network in synthetic biology.

More information is available on:
www.synbio-symposium.de

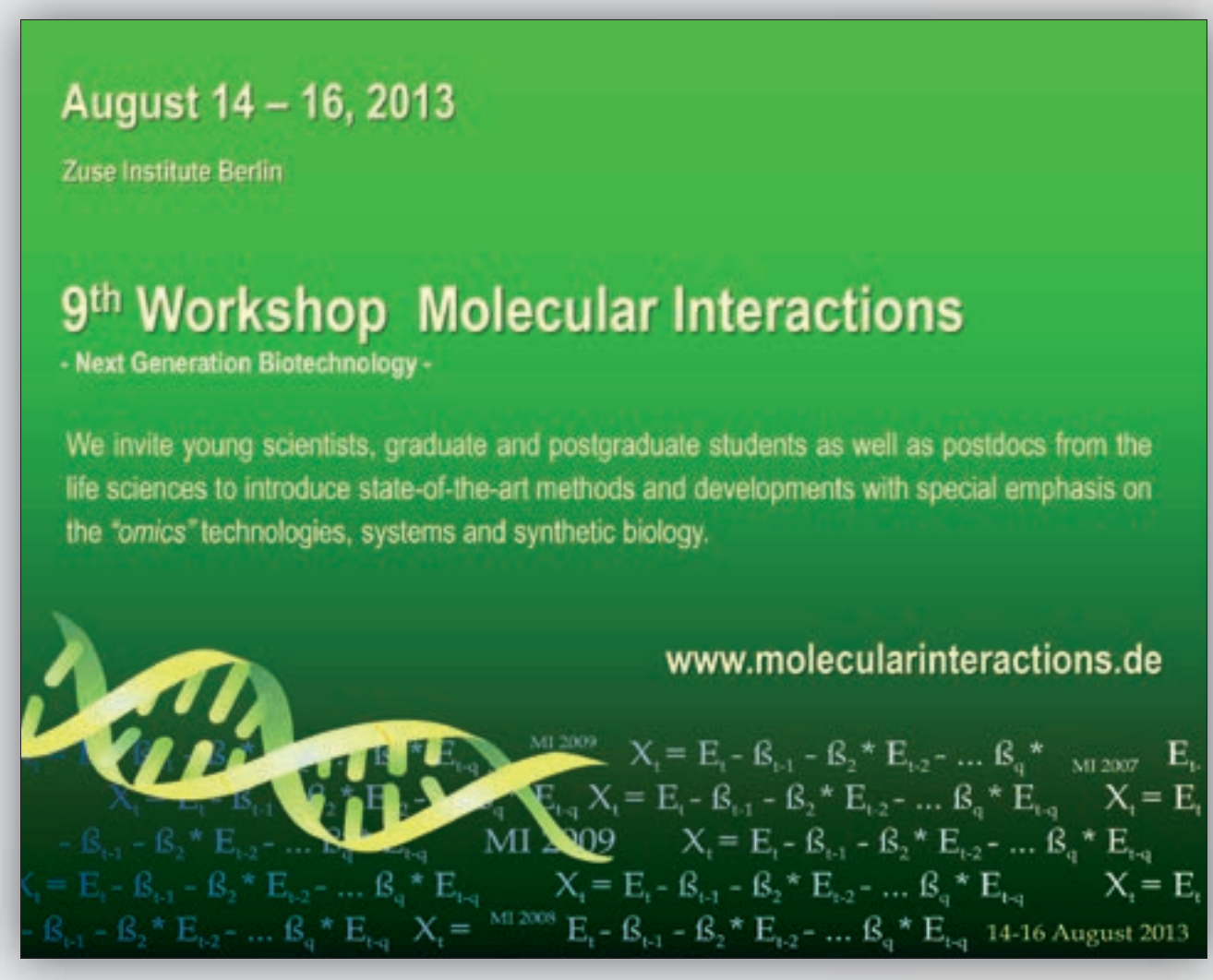
9th Workshop Molecular Interactions – Next Generation Biotechnology

August 14 – 16, 2013, Zuse Institute Berlin, Germany

The Workshop Molecular Interactions will be held for the 9th time in Berlin (Germany) with the focus on "Next Generation Biotechnology". Invited talks from experts from Germany and Europe on "omics" research, Systems Biology and Synthetic Biology will present the state-of-the-art methods and developments in the respective fields. The motivation of the workshop is to give an insight into the most recent trends, methods, technologies and analytical approaches in the life sciences. We invite young scientists, graduate and postgraduate students as well as postdocs from all life science areas to discuss, exchange and network. The scientific presentations will be complemented by a professional information event introducing young scientists to different career opportunities. The workshop will take place at the Zuse Institute Berlin from August 14 - 16, 2013.

For more information and registration please visit our website:

www.molecular interactions.de



Conference report

EU Science: Global Challenges – Global Collaboration

March 4 - 8, 2013, Brussels, Belgium

A five-day conference on "EU Science: Global Challenges, Global Collaboration" took place in the European Parliament in Brussels. The conference brought together science policy-makers, scientists and industry representatives from 100 different countries and placed EU research at the centre of the international response to global challenges. It was convened by ISC - Intelligence in Science, in association with the Irish Council Presidency together with Sean Kelly and other MEPs. Centrepiece of the conference was the 50 years roadmap for the Future in Medicine (www.globalsciencecollaboration.org).

To achieve long-term sustainability of health care systems, substantially new concepts are required which have to be based on sound science and can only be achieved by inter-disciplinary and international collaborations. Also business models will undergo fundamental changes; this issue was discussed in a session chaired by Angela Brand (Maastricht University) and Babette Regierer (LifeGlimmer GmbH).

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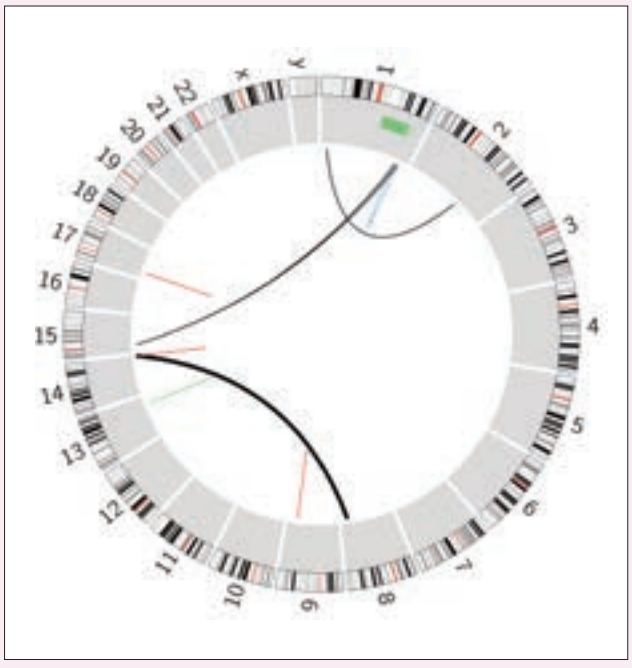
news

German contributions to the International Cancer Genome Consortium reveal important mechanisms in cancer development

The International Cancer Genome Consortium (ICGC) aims at providing a comprehensive catalog of mutations in the 50 most important cancer types. Starting from 2007, up to 25,000 tumor samples will be studied. Germany is contributing to this consortium with three independent projects, which now report on their first results. They target brain tumors in children, prostate cancer in men under the age of 50, and malignant Non-Hodgkin lymphoma. The German Cancer Research Center (DKFZ), together with the European Molecular Biology Laboratory (EMBL), the Max Planck Institute of Molecular Genetics, and the University of Leipzig, had a leading role in the data analysis of these tumor genome sequence data.



Changes in the genome of a Burkitt lymphoma. In addition to the characteristic Burkitt translocation between chromosome 8 and chromosome 14 (thick black line), the genome shows further alterations: genetic material between two chromosomes is exchanged (translocation: black), inverted (inversion: blue), additionally inserted (insertion: green) or removed (deletion: red) (Source: M. Schlesner).

The German ICGC project PedBrain studied the genome of 125 **medulloblastomas** by means of high-throughput sequencing (Jones *et al.*, 2012). The number of somatic mutations is increasing with age at diagnosis, but the total number is still far below that typically found in cancers of adults. A significant fraction of somatic mutations hits genes that are involved in gene regulation by epigenetic modifications. About one in three tumors shows four instead of two copies of each chromosome. This tetraploidy may provide an opportunity to fight these cancers by drugs that target cells with more than the usual number of chromosomes. In a second study, the researchers could demonstrate that the newly discovered phenomenon of chromothripsis (chromosome shattering) is linked – at least in medulloblastoma – to inactivation of the p53 protein (Rausch *et al.*, 2012).

Prostate carcinoma is diagnosed on average at an age of about 70 years. Since it is in many cases progressing very slowly, elderly patients often do not require any aggressive treatment. About 2 % of all patients, however, are diagnosed with prostate cancer already at an age of 50 years or even younger. The ICGC consortium on early-onset prostate carcinoma has now sequenced and analysed the genomes of tumors from 11 such patients (Weischenfeldt *et al.*, 2013). The bioinformatic analysis demonstrated that young patients harbor a higher fraction of genome aberrations that depend on the action of the androgen receptor. This has been corroborated in a validation cohort of more than 10,000 patients.

The ICGC project on malignant lymphoma has sequenced the complete genome sequence of four **Burkitt lymphoma** samples arising in children (Richter *et al.*, 2012). One of the recurrently mutated genes is ID3 (inhibitor of DNA binding 3), which has been inactivated in two cases by mutations on both alleles of this gene (inherited genome copies from the parents). Targeted analysis of 100 other B cell lymphoma demonstrated that ID3 is mutated in 68 % of all Burkitt lymphoma, but in very few of the other lymphoma subtypes. Thus, ID3 would be a promising target for selective therapies, and can be used as a marker to diagnose Burkitt lymphoma, which proves to be difficult.

Publications:

Jones DTW*, Jäger N* *et al.* (2012) Dissecting the genomic complexity underlying medulloblastoma. Nature 488:100-105.

Rausch T*, Jones DTW*, Zapatka M*, Stütz A* *et al.* (2012) Genome sequencing of pediatric medulloblastoma links catastrophic DNA rearrangements with TP53 mutations. Cell 148:59-71.

Richter J*, Schlesner M*, Hoffmann S*, Kreuz M*, Leich E*, Burkhardt B* *et al.* (2012) Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. Nat. Genet. 44:1316-1320.

Weischenfeldt J*, Simon R*, Feuerbach L*, Schlangen K*, Weichenhan D*, Minner S*, Wuttig D* *et al.* Integrative genomic analyses reveal androgen-driven somatic alteration landscape in early-onset prostate cancer. Cancer Cell, 23:159-170.

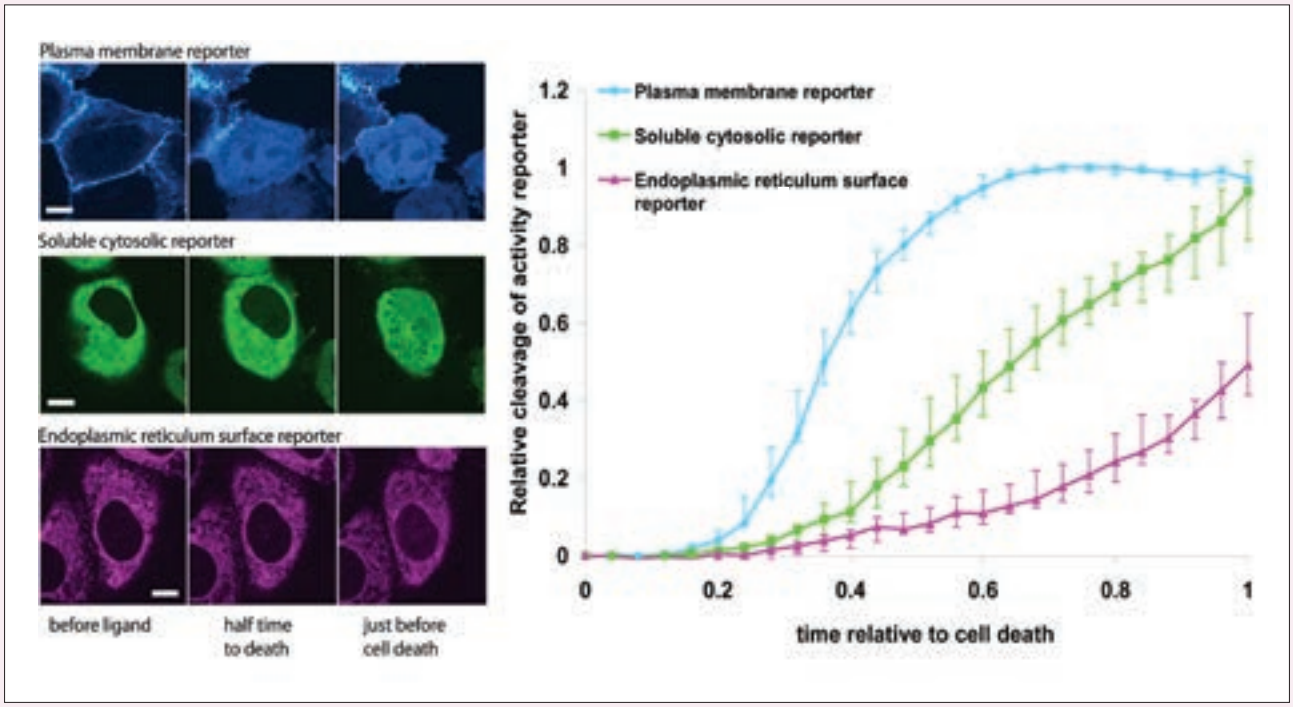
(*shared first authorship)

Source: DKFZ Heidelberg

Publication in Cell Death and Differentiation: Changing the model of CD95 induced apoptosis with a new tool

The Signal Transduction Biophysics group, part of the eilslabs at DKFZ Heidelberg, has recently published a paper describing an innovative method to measure spatial and temporal activity of caspases in living cells. The group of Joel Beaudouin has developed reporter proteins that combine a fluorescent protein and an intracellular location signal connected through a caspase cleavage sequence. Activity was measured by quantifying fluorescent protein cellular redistribution upon separation from its localization. By combining different localization domains, fluorescent proteins and cleavage linkers, one can measure within the same cell activity from different caspases at different cellular compartments.

With this new tool it was possible to get new insights into the CD95-induced apoptotic cascade. Up to now it was thought



Membrane-bound reporters are cleaved more efficiently than soluble cytosolic reporters. The latter are cleaved more efficiently than reporters that have no access to the cell membrane, e.g. on the surface of the ER. Shown are HeLa-cell overexpressing the CD95 receptor. Scale 10µm (Source: Joel Beaudouin).

that caspase 8 needs to be released into the cytosol in order to cleave its substrates Bid and caspase 3. However, Beaudouin and his co-authors could show that caspase 8 is already fully active and can cleave its substrates on the cell membrane in order to trigger programmed cell death. The new sensors provide a quantitative tool that will allow modelling initiation and propagation of caspase activity inside the cell depending on the concentration and distribution of the different proteins involved in the apoptotic pathway.

Publication:

Beaudouin J, Liesche C, Aschenbrenner S, Hörner M and Eils R (2013) Caspase-8 cleaves its substrates from the plasma membrane upon CD95-induced apoptosis. Cell Death and Differentiation 20:599-610.

Source: Clarissa Liesche

CASyM and the road to systems medicine

In November 2012, the Directorate of Health of the European Commission launched the large scale Coordinating Action Systems Medicine – CASyM to develop a roadmap outlining an integrative strategy for the implementation of systems medicine across Europe. Main driver of the proposed road map are clinical needs - from clinical trials through public health and data handling, to application of systems medicine and medical economics.

An open networking concept based on professional conferences, workshops and forums involving high-profile stakeholders from the clinical sector, academia, industry, government, and patient organizations across Europe is CASyM´s key to integrate all relevant stakeholders and become the European umbrella concept for systems medicine.

CASYM EUROPE | Coordinating Action Systems Medicine
Implementation of Systems Medicine across Europe

CASyM initiated this process with its first comprehensive stakeholder conference in Lyon in March 2013 as a satellite event of the Biovision World Forum on Life Sciences. This initial stakeholder conference covered a wide range of different topics from lifelong education through technological details and data handling issues, from ethical/regulatory issues and patient´s care/involvement through business models for industry and from clinical trials and mathematical modelling through impact indicators of systems medicine efficiency.

Approximately 150 experts discussed these topics intensively at ten moderated round tables. The output of each round table discussion was a set of specific chapters describing (i) state of the art, (ii) challenges, (iii) opportunities, and (iv) implementation strategies for each topic. These round table chapters will be an integral part of the proposed road map for systems medicine, which CASyM will produce in the coming year.

CASyM continued its road map process with a follow up conference in St Andrews in May 2013. This event was thematically more focused and specifically investigated examples and questions of real medical problems in smaller series of round table

imprint

discussions. Besides, CASyM will also organize a series of focussed workshops in 2013, explicitly targeting other key aspects of the road map to systems medicine.

Please visit www.casym.eu for more information and subscribe to the CASyM newsletter.

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Source: Projekträger Jülich

e:Med: Paving the way for systems medicine

In order to strengthen systems oriented approaches in studying human diseases and prevention, the research and funding concept e: Med is launched by the Federal Ministry of Education and Research (BMBF). This concept focuses on interdisciplinarity, medical application, promotion of young scientists, cross-sectional and future issues as well as internationalisation.

With the now announced guidelines for funding 'Demonstrators for Individualised Medicine', module 2 of the future concept e: Med has been implemented.

Demonstrators are pilot projects. They should demonstrate the direct benefits and applicability of results from basic research in the field of individualised medicine. In particular, systems-oriented medical approaches for individualised prevention, diagnostics, and treatment of human diseases should be applied. In interdisciplinary research groups from different theoretical (physics, mathematics and computer science) and practical disciplines (life sciences and different clinical disciplines), life sciences and information sciences are brought together in a way that a systems oriented view of diseases is possible. With these funding guidelines, the BMBF makes a contribution to the field of action 'Individualised Medicine' in the Federal government's Health Research Framework.

Find more on:
www.ptj.de/demonstratoren
Source: Projektträger Jülich

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about us

Presenting the systembiologie.de editorial team

systembiologie.de would like to make the success of German systems biology accessible to a wider public in an illustrative way. The magazine, which is published twice per year in German and once in English, is produced jointly by the offices of the national systems biology networks Helmholtz Alliance on Systems Biology, Helmholtz Initiative on Synthetic Biology, Virtual Liver Network, and Projektträger Jülich.

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- from understanding to application -

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German Cancer Research Center (DKFZ)
Heidelberg, Germany

Confirmed Invited Speakers:

Luke Alphey
University of Oxford (UK)

Michelle Chang
University of California, Berkeley (USA)

John Glass
J. Craig Venter Institute (USA)

Jeff Hasty
University of California, San Diego (USA)

Petra Schwille
Max Planck Institute for Biochemistry, Martinsried

Eckard Wimmer
Stony Brook University, NY (USA)

www.synbio-symposium.de

Organizing Committee:

Roland Eils
German Cancer Research Center, Heidelberg
Heidelberg University

Stefan Bräse
HEiKA - Heidelberg-Karlsruhe Research Partnership

Christopher Coenen
Karlsruhe Institute of Technology

Kristian Köchy
Berlin-Brandenburgische Akademie der Wissenschaften

Harald König
Karlsruhe Institute of Technology

Ulrike Protzer
Helmholtz-Zentrum München

Michael Reth
BIOSS - Center for Biological Signalling Studies, Freiburg

Uwe Strähle
Karlsruhe Institute of Technology

Wilfried Weber
BIOSS - Center for Biological Signalling Studies, Freiburg
NSB - Upper Rhine network in synthetic biology

Wolfgang Wiechert
Research Center Jülich
DECHEMA

Dagmar Wirth
Helmholtz-Zentrum for Infection Research, Braunschweig

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Helmholtz Initiative on Synthetic Biology

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