BioSysNet

Bavarian Research Network for Molecular Biosystems

Molecular Biosystems

– How is life organized?

Imprint

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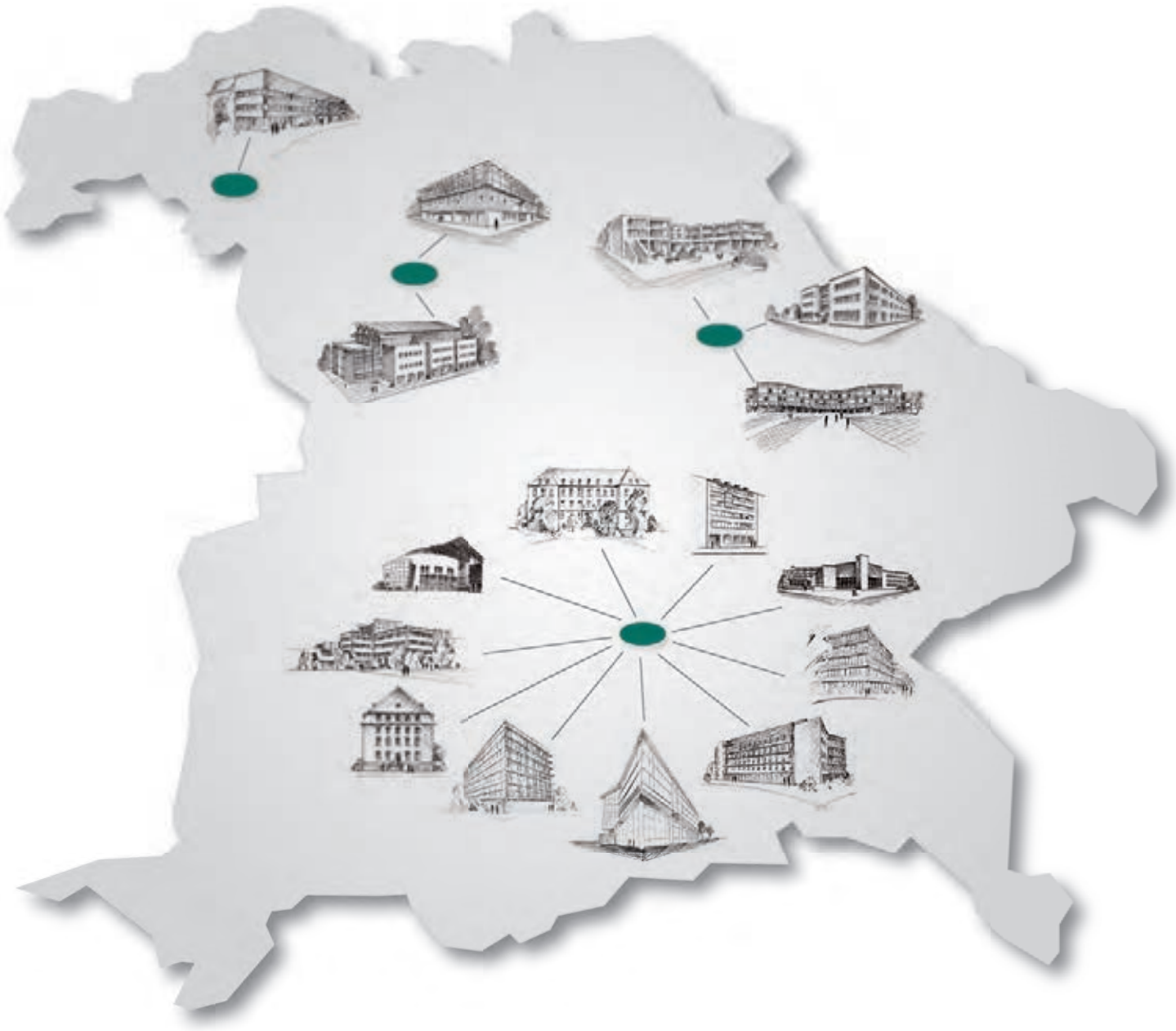


Content

Greeting

Dr. Wolfgang Heubisch Bavarian State Minister for Sciences, Research and the Arts 6

Prof. Dr. Horst Domdey Coordinator of BioSysNet 7

Prof. Dr. Patrick Cramer Speaker of the Strategic Council of BioSysNet 8

BioSysNet

Managing Director and Head Office of BioSysNet Introducing BioSysNet 9

Regular Junior Groups

Dr. Fabiana Perocchi Functional Genomics of Mitochondrial Physiology 12

Dr. Olaf Groß Molecular Mechanisms and Effects of Inflammasome Activity 14

Dr. Tobias Madl Bringing Order to Protein Disorder 16

Dr. Jan Medenbach Translational Control in Eukaryotes 18

Dr. Ana Eulalio RNA: The Missing Link in Bacterial Pathogen-Host Interactions 20

Associated Junior Groups

Dr. Beate Winner Transcriptome Analysis to Delineate Genes Involved in Synaptic Dysfunction in Synucleinopathies 22

Prof. Dr. Ulrich Gerland Physical Analysis of Nucleosome Positioning, Remodeling, and Transcription Regulation in Yeast 23

Prof. Dr. Mario Halic Small RNA Mediated Heterochromatin Formation in Fission Yeast 24

Dr. Philipp Korber Reconstitution of Nucleosome Positioning Mechanisms for *S. cerevisiae* and *S. pombe* 25

Dr. Johannes Söding Analysis and Modeling of Regulatory Protein-DNA Binding Energy Landscapes 26

Prof. Dr. Dr. Fabian Theis How Stem is a Stem Cell? 27

Prof. Dr. Gil Westmeyer Spatiotemporal Control of mRNA Levels 28

Dr. Cynthia Sharma Exploring RNA-binding Proteins in *Campylobacter jejuni* 29

Associated Senior Groups

Prof. Dr. Robert Slany	Transcriptional Priming as Global Mechanism Controlling Self Renewal and Differentiation during Hematopoietic Development	30
Prof. Dr. Karl-Peter Hopfner	Molecular Systems Analysis of Virus Genome Sensing	31
Prof. Dr. Christoph Klein	A Systems Biology Perspective on Pediatric Inflammatory Bowel Diseases	32
Prof. Dr. Andreas Ladurner	Gene-Environment Interactions	33
Prof. Dr. Eckhard Wolf	Developmental Impact of Maternal Diabetes Mellitus	34
Prof. Dr. Ralf Zimmer	Exploring and Explaining Expression Patterns	35
Prof. Dr. Dr. Stefan Engelhardt	Cooperative miRNA Function in Cardiovascular Disease	36
Prof. Dr. Anja Bosserhoff	Molecular Understanding of Malignant Melanoma - Lessons from Embryogenesis	37
Prof. Dr. Gunter Meister	Proteomics-Based Identification of Cellular Networks Regulating miRNA Maturation	38
Prof. Dr. Rainer Spang	Modelling Paracrine Communication	39
Prof. Dr. Jörg Vogel	Temporal Control of Gene Expression by Small RNAs	40

Foundations

Prof. Dr. Christoph Klein	Care for Rare Foundation	41
Dr. Beate Winner	Tom Wahlig Foundation	42

Cooperation Partners in the Industry 43

Greeting

Dr. Wolfgang Heubisch
Bavarian State Minister for Sciences, Research and the Arts

Contact
Bavarian State Ministry
for Sciences, Research
and the Arts,
Munich

BioSysNet - The Bavarian Research Network for Molecular Biosystems

Research in life sciences and biotechnology is already extraordinarily high-performing in Bavaria. With the Bavarian Research Center for Molecular Biosystems (BioSysNet) and a new Core Center at Ludwig-Maximilians-University München (LMU Munich), we want to make the Free State even more competitive in innovative biosystems research and further strengthen its international visibility. For this, we have allocated around 18.1 million euros within the framework of our initiative for the future "Aufbruch Bayern". Furthermore, we will invest 13.65 million euros in building a research facility for molecular biosystems at LMU Munich.

BioSysNet aims at solving one of the central problems of life sciences: the regulation of gene expression. On the basis of these findings, new diagnostic methods and personalised therapy approaches can be developed. This challenge can only be met if experts from different disciplines collaborate – especially from biochemistry, genetics, bioinformatics, biophysics and medicine. As a consequence, BioSysNet functions as a true network bringing together competencies within our Free State and therefore also attracting renowned scientists from abroad to Bavaria.

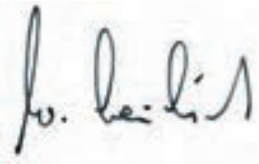
This is why, besides scientific excellence and disciplinary diversity, international experience has also played a role in the selection of the five new independent junior research groups. I am delighted that we have succeeded in attracting excellent young scientists from renowned European research institutions to BioSysNet and the involved universities.

The network is complemented by the expertise of already established scientists, who receive additional funding for their projects within BioSysNet as so-called associated junior research groups or associated senior research groups.

The exchange between members of the network will contribute to better understanding biological systems on a cellular and molecular level and thus further advancing top biosystems research in Bavaria.

I wish you an inspiring read of the following pages that will inform you about the contents of the funded research projects, the network structure, and the schedule of events.

Munich, June 2013



Dr. Wolfgang Heubisch

Greeting

Prof. Dr. Horst Domdey
Coordinator of BioSysNet

Interdisciplinary Research in the Bavarian Landscape - Biosystems

The field of molecular biosystems is one of the most innovative and fast developing research topics of the 21st century. It has become a driving force in biological and medical research and is by nature and has to be in practice a truly interdisciplinary approach.

The newly founded Bavarian Research Network for Molecular Biosystems connects scientific groups from all over Bavaria. Scientists at different institutes and departments are investigating interdisciplinary topics of Molecular Biosystems to create new insights into the interconnectivity of seemingly separated fields in cellular and organismal as well as biophysical sciences, informatics and mathematics. The strategy behind this program is to concentrate the competencies in this scientific subject and strengthen the position of the Free State of Bavaria in this highly competitive area at the global level. For the future, a combined molecular biosystems approach will provide new insights and possibilities for diagnoses and the treatments of diseases. There are already great efforts in the field of "personalized medicine" in Bavaria. Interacting with this program and other Bavarian Research Networks the funded projects in basic research will give new prospects and knowledge to improve peoples' health - an important economic sector for Bavaria and Germany.

Intensive cooperation with scientific groups abroad is well established and will help us to maintain new developments. By this we combine the strength of Bavaria with the international leaders and will keep up with the competitors in the field of Molecular Biosystems.

An international approved scientific committee selected the best applicants in a very competitive selection process. "Excellence" of the projects and the scientists was the only criteria that counted. We are more than happy to foster 19 excellent scientific heads in Bavaria and at the same time, to invite five junior scientists to build up their new project groups in our scientific landscape.

We have to thank the Bavarian Government, especially the Bavarian State Ministry of Sciences, Research and the Arts, to realize this kind of combined and out-standing research. I hope you enjoy reading this brochure and find it an exciting and stimulating resource.

Munich, June 2013

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Greeting

Prof. Dr. Patrick Cramer
Speaker of the Strategic Council of BioSysNet and Chair of the Bavarian Research Center for Molecular Biosystems BioSys^{M}

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Gaining the future in biomedicine

Thank you for your interest in BioSysNet, the Bavarian Research Network for Molecular Biosystems!

The life sciences are entering a new era. In the past, we studied individual genes and gene products, leading to a detailed understanding of many mechanisms underlying the function of living cells in health and disease. Today, new systemic methods extend the classical approaches. They enable us to sequence entire genomes, to identify most gene products in living cells, and to map interaction networks between components of living systems. There is now a great opportunity at the interface of classical molecular biology and recent systemic approaches. A new research field develops that we call molecular systems biology or molecular biosystems research.

This new research field requires a high degree of interdisciplinarity. Geneticists, molecular biologists, and strutural biochemists must interact closely with mathematicians and bioinformaticians. This allows us to combine classical approaches with modern systemic methods to gain insights into the inner workings of living cells, eventually unravelling the molecular mechanisms that generate and maintain living systems. Of particular interest here is the long-standing question of how the information in the genome is used. What are the mechanisms that ensure that the right sets of genes are activated and silenced at the right place and at the right time? To answer this fundamental and complex question, molecular biosystems research relies on new frameworks that foster interaction and scientific exchange.

In this brochure we present the newly established Bavarian research network for molecular biosystems. The network brings together 24 excellent junior and senior research groups at four locations in Bavaria. It is part of a larger effort to strengthen molecular biosystems research in Bavaria by establishing a Bavarian Research Center for Molecular Biosystems. Contributing to this goal, a new research building is currently under construction on the Munich life science campus Großhadern-Martinsried. In addition, a core center at the Ludwig-Maximilians-Universität München will establish lacking expertise by recruiting new research groups and will provide infrastructure support. Together these measures will foster technical advances, cutting-edge research and innovations, and enable us to train the next generation of life scientists.

This brochure provides an overview of the work conducted within our new research network. It impressively demonstrates the scientific excellence of the project leaders and a wealth of scientific projects. I wish all colleagues involved best of luck and breakthrough results. Let us together gain the future in biomedicine!

Munich, June 2013

Prof. Dr. Patrick Cramer

Introducing BioSysNet

Managing Director and Head Office of BioSysNet

One network throughout Bavaria

Leading new ways in molecular systems biology for a better understanding of functions and diseases.

► During recent years the Bavarian State government has made a great effort to strengthen research, innovation and technology in Bavaria. One remarkable success story is the Bavarian Genome Research Network, funded in 2004. Based on its great achievements, the Bavarian State Ministry of Sciences, Research and the Arts established the Bavarian Research Network for Molecular Biosystems at the end of 2011. The objective of the new network is to bundle local expertise in systems biology and thus create ideal research conditions for the scientists working in this program. Outstanding research groups from different universities throughout Bavaria work together in order to obtain a holistic view on the regulation of living cells. The Bavarian Research Network for Molecular Biosystems under the scientific coordination of Professor Dr. Horst Domdey (BioM) is part of the Bavarian Research Center for Molecular Biosystems chaired by Professor Dr. Patrick Cramer (BioSys^{M}). A scientific advisory board, consisting of internationally renowned experts, will accompany and regularly evaluate the work of the network.

Groups within the network

► Systems biology is currently one of the most dynamic and trendsetting areas in biological research. In order to support further innovative projects in this field, the Bavarian government funds cutting edge research projects on the platform of BioSysNet. Therefore the network supports both new, independent junior research groups as well as established junior and senior research groups in their joint quest to gain a better understanding of molecular biosystems. The program is split up into two different funding schemes. First of all, five international and young scientists are given the opportunity to build up an independent and new research group in the Free State of Bavaria. The new groups are located at the Julius-Maximilians Universität Würzburg (Dr. Ana Eulalio), at the Ludwig - Maximilians - Universität München (Dr. Fabiana Perocchi), at the Technische Universität München (Dr. Tobias Madl and Dr. Olaf Groß) and at the Universität Regensburg (Dr. Jan Medenbach). The newly recruited regular junior group leaders receive funding of up to 1.5 million euros over a period of five years. The funding program enables those talented and ambitious young scientists to develop their own independent research programs.

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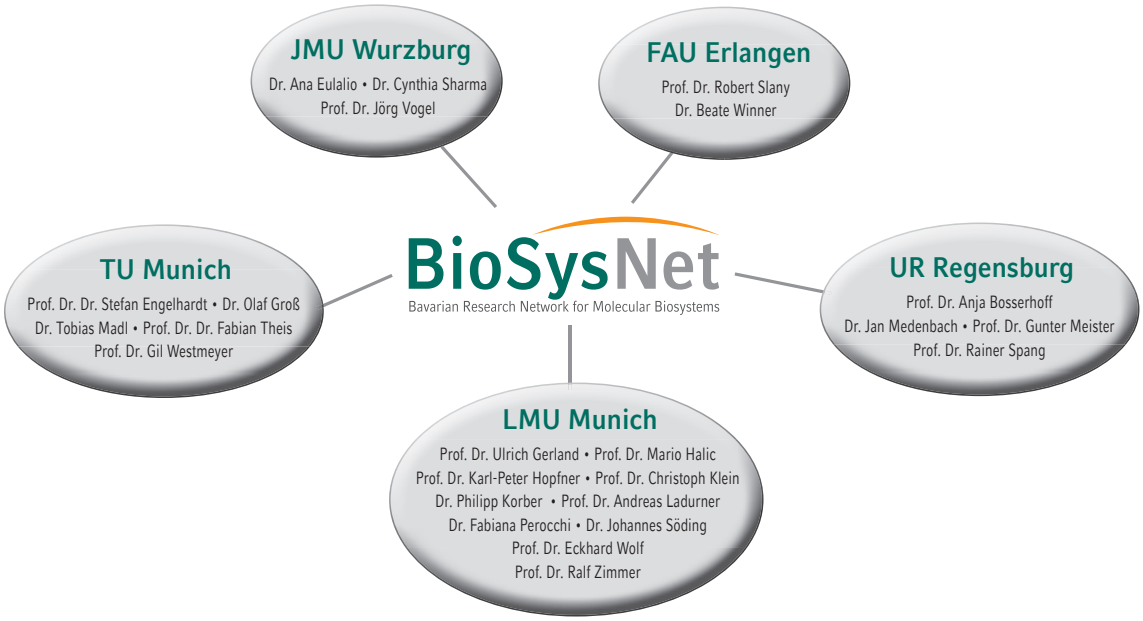


The different groups within the Bavarian Research Network for Molecular Biosystems.

BioSysNet

The Bavarian Research Network for Molecular Biosystems

In addition, 19 associated and well established research groups in Bavaria will receive additional funding of up to 250.000 euros, also over a period of five years. This program enables them to follow up on interesting project ideas and develop new relevant technologies.

All groups conduct their research independently. An essential component of the network is the enhanced potential for the exchange of ideas and expertise between its members. Progress seminars and symposia provide a basis for scientific collaboration and mutual support. All funded groups are closely associated with their host institutions, but they are also members of the Bavarian Center for Molecular Biosystems.

Different facilities combined under one roof

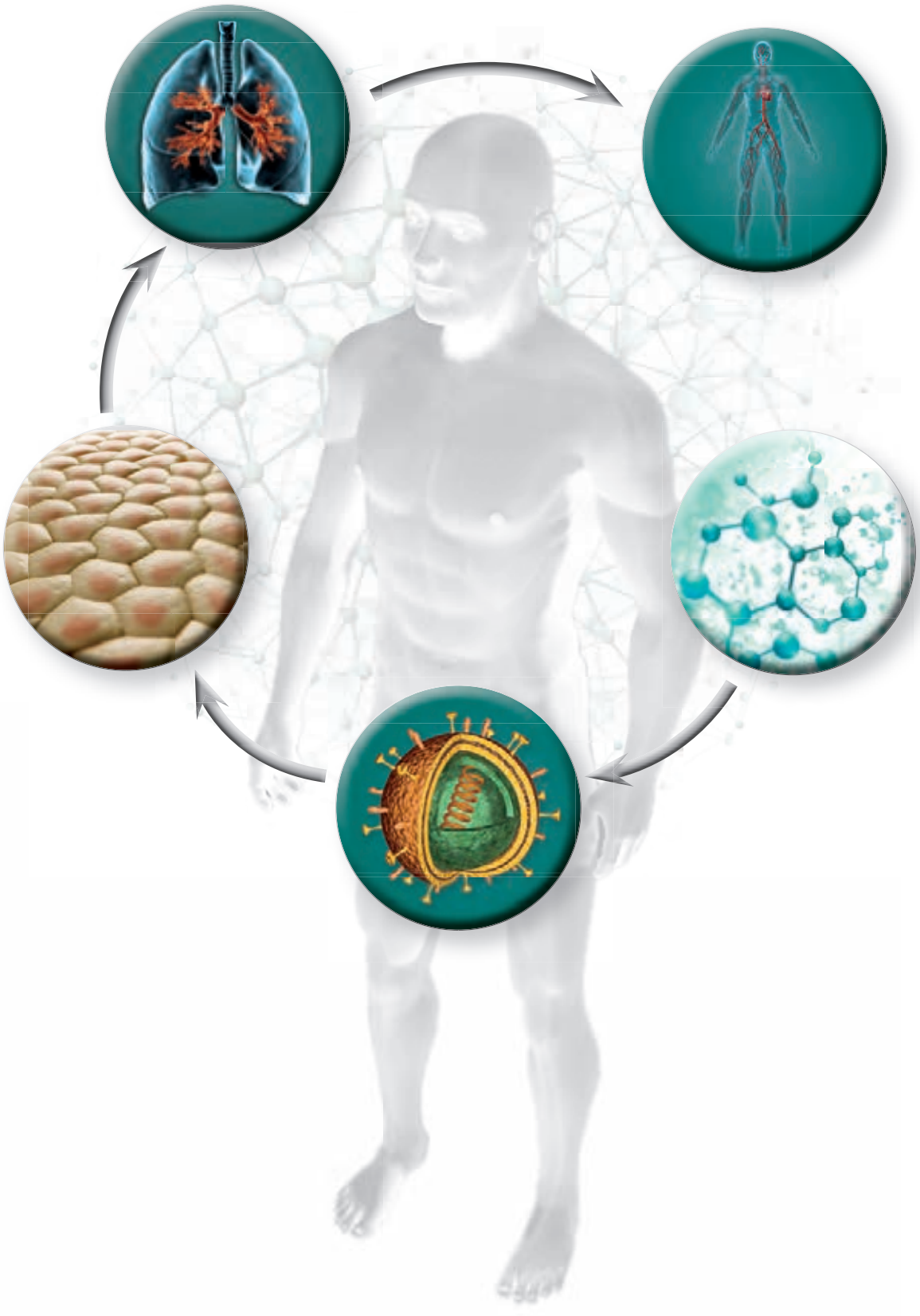
► Developments over recent years has shown that genome research is focusing increasingly on the behavior of complex systems. Therefore, BioSysNet will address various scientific questions: How is gene expression regulated? Which are the components of a biological system? How do these components interact with each other? How does the system react as a whole, when left to itself or when disturbed?

Answering these questions has always been one of the central interests of the life sciences, but only nowadays has it become possible to study components, interactions and perturbations on a molecular level with high quantitative definition. To achieve a holistic view, systems biology research has to cross academic and institutional boarders. Over all BioSysNet aims at understanding the function and malfunction of cells and organisms or even their malfunction in diseases such as cancer and neurodegenerative disorders.

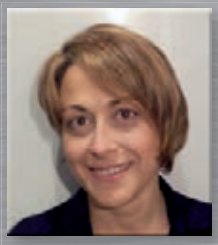
This interdisciplinary undertaking integrates research projects from different areas such as biology, biochemistry, chemistry, medicine, physics and mathematics. The continuous development of important new technologies plays a central role, as well as the interpretation of complex interactions with already established analytical methods. The discussion of results within the network will significantly improve the processing time of scientific assessments.

Outlook

► Research in life sciences and biotechnology is already extraordinarily high-performing in Bavaria. Even today, it plays a leading role in the European research landscape. The new network will help the federal state to keep pace with the international competition, as a rapid development in this field is initiated. BioSysNet supports the high-tech location of Bavaria for the successful implementation of new research techniques and innovations. The profile of the scientific and educational environment is strengthened and thus ensures a future oriented alignment of the State of Bavaria, which is a front-runner on the area of functional genome research compared with other European countries. In order to enhance this status, the knowledge and human resources for the formation of a new generation of companies and the extension of existing ones have to be promoted. Only in this way can the dynamic development of the Bavarian biotechnological industry be ensured.



Molecular Biosystems: From molecular scale to cells, tissues, organs and the entire human body. Unterstanding functions and diseases on a whole new level.



Functional Genomics of Mitochondrial Physiology

Systematic Reconstruction of Mitochondrial-Cellular Crosstalk

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Introduction

► Mitochondria are double membrane organelles found in virtually all eukaryotic cells. They serve as the center-stage for energy production, ion homeostasis, intermediary metabolism, and cell death. Mitochondria have a special evolutionary origin, derived from bacteria engulfed about 2 billion years ago by eukaryotes. Several mitochondrial functions have been retained over evolution, so that unicellular yeast and multicellular organisms share many of the same mitochondrial components [1,2] (Fig. 1).

Human mitochondria contain a tiny genome encoding 13 well-studied proteins, while all remaining estimated 1500 mitochondrial proteins are encoded from nuclear genes, translated in the cytosol, targeted and imported into the organelle. As a consequence of the dual genetic origin of mitochondrial proteins, mitochondrial biogenesis and function require extensive crosstalk between cellular and mitochondrial machineries (Fig. 1). Bidirectional signaling exists between mitochondria and other cellular components that regulates the dynamic and complex remodeling of mitochondrial shape, motility, metabolism, and proteome in response to environmental stimuli and energetic requirements during growth and development. However, in many instances, the molecular links between intracellular signals and mitochondrial responses remain unknown [3,4]. How do mitochondria integrate into signal transduction cascades? What are the mitochondrial proteins that sense, modulate, and propagate intracellular signals? How is the mitochondrial signaling "toolkit" regulated? What happens when mitochondrial signaling networks are compromised and how can we prevent deleterious effects?

Goals of the project

► The long-term goal of our laboratory is to achieve a systems-level understanding of human mitochondria to advance basic and disease biology [2] (Fig. 2). Specifically, we aim at identifying the molecular machines and mechanisms responsible for calcium signaling in mitochondria. To this goal, we combine large-scale, computational and experimental strategies with focused genetic,

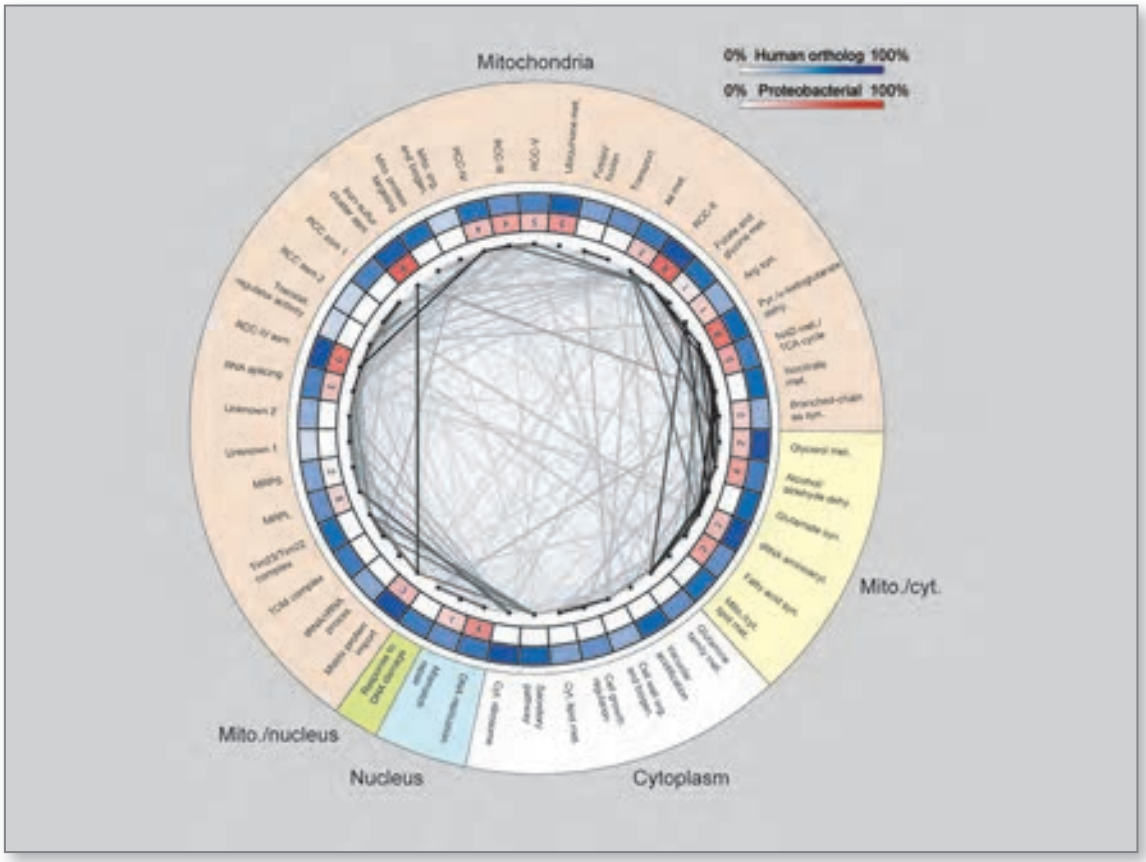

Figure 1: *Functional network of the yeast mitochondrial system: origin, conservation, and crosstalk. From Perocchi et al., PloS Genetics 2006.*

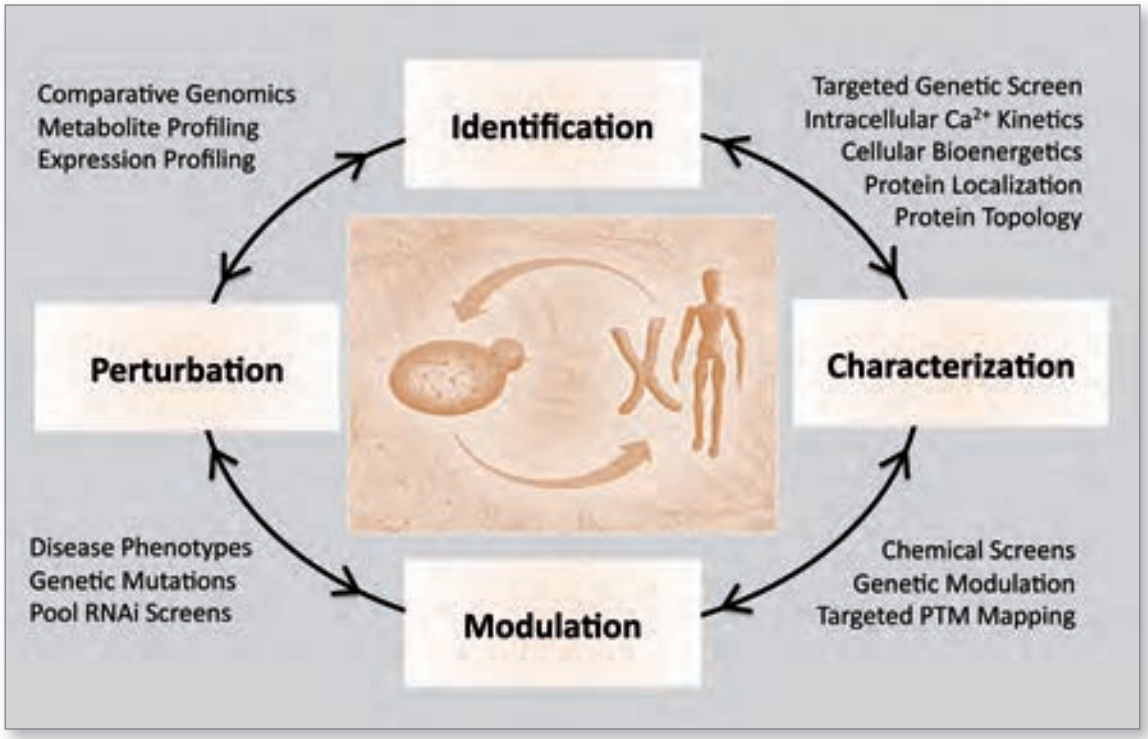

Figure 2: *Schematic summary of the integrative approach used to reconstruct mitochondrial signaling networks.*

biochemical, and physiological studies of mitochondrial functions in fungi, mouse, and human cells. We develop high-throughput assays to test and reverse the effects of genetic and chemical perturbations on mitochondrial calcium signaling and to spotlight key signaling checkpoints that could be targeted pharmacologically. Comprehensive and quantitative measurements of metabolites, proteins, and their post-translational modifications will be performed to decipher the mitochondrial calcium footprint on cell metabolism and protein regulation. In light of the fundamental roles of calcium signaling in development, learning, metabolism, and neuromuscular function, and the ability of mitochondria from virtually all tissues to regulate calcium homeostasis, we hope our research will have implications for understanding the pathological mechanisms of several human disorders.

Conclusions and outlook

► Given the key role of mitochondria in cell physiology, our research has far-reaching implications in human health and disease. Mitochondrial dysfunction is linked to virtually every age-associated disease, including diabetes, cancer, and neurodegeneration. Several genetic studies have implicated genes that encode mitochondrial proteins (e.g. PINK1, PARK2, and DJ-1 in familial Parkinson's; L2HGDH in gliomas). As more mitochondrial genes are implicated by whole-exome sequencing studies, our functional characterization of mitochondrial signaling pathways will provide valuable mechanistic insights and new perspectives for molecular intervention.

Selected Publications

1. Perocchi F, Jensen LJ, Gagneur J, Ahting U, von Mering C, Bork P, Prokisch H, Steinmetz LM (2006). Assessing systems properties of yeast mitochondria through an interaction map of the organelle. PLoS Genetics. 2(10):e170.

2. Perocchi F*, Mancera E*, Steinmetz LM (2008). Systematic screens for human disease genes, from yeast to human and back. Molecular Biosystems. 4(1):18-29.

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5. Gohil VM*, Sheth SA*, Nilsson R, Wojtovich AP, Lee JH, Perocchi F, Chen W, Clish CB, Ayata C, Brookes PS, Mootha VK (2010). Nutrient-sensitized screening for drugs that shift energy metabolism from mitochondrial respiration to glycolysis. Nature Biotechnology. 28(3):249-55.

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Molecular Mechanisms and Effects of Inflammasome Activity

Using proteomics to gain a better understanding of the molecular mechanisms of inflammation

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Introduction

► Inflammasomes are intracellular danger-sensing protein complexes that are important for host protection through the initiation of inflammation. The two major effects of inflammasome activity are the maturation and secretion of IL-1 family cytokines and the initiation of a certain form of cell death, called pyroptosis. Both are mediated by the common component of all inflammasomes, the protease caspase-1. Inflammasomes and IL-1 family cytokines are present in our body in certain types of immune cells including macrophages, the major phagocytic cell type of the immune system that is responsible for pathogen uptake and destruction. The two IL-1 family cytokines IL-1α and IL-1β induce local and systemic reactions including inflammation and fever, thereby playing a crucial role in host defence. The function of pyroptosis is to remove the niche of pathogens that reside within innate immune cells, and thus expose them to destruction by the immune system.

On the molecular level, inflammasomes are cytoplasmic multi-protein complexes that contain a danger sensing molecule connected to caspase-1 through interactions with the adaptor protein ASC. To date, five inflammasome-activating sensor proteins have been described in some detail: NLRP3, NLRP1, NLRC4, AIM2, and RIG-I. Formation of the latter four inflammasomes is promoted by specific stimuli. While the NLRP1 and NLRC4 inflammasomes are activated by microbial stimuli derived from anthrax-causing bacteria or Salmonella species, respectively, presence of viral DNA or RNA in the cytoplasm activates the AIM2 and RIG-I inflammasomes. In contrast, the NLRP3 inflammasome is activated in response to severe cellular stress that can be induced by a multitude of physically and chemically dissimilar triggers of endogenous and exogenous origin. Through this wide spectrum of activators, inflammasomes are key in sensing the presence of danger to the cells of our body and the activation of an inflammatory response.

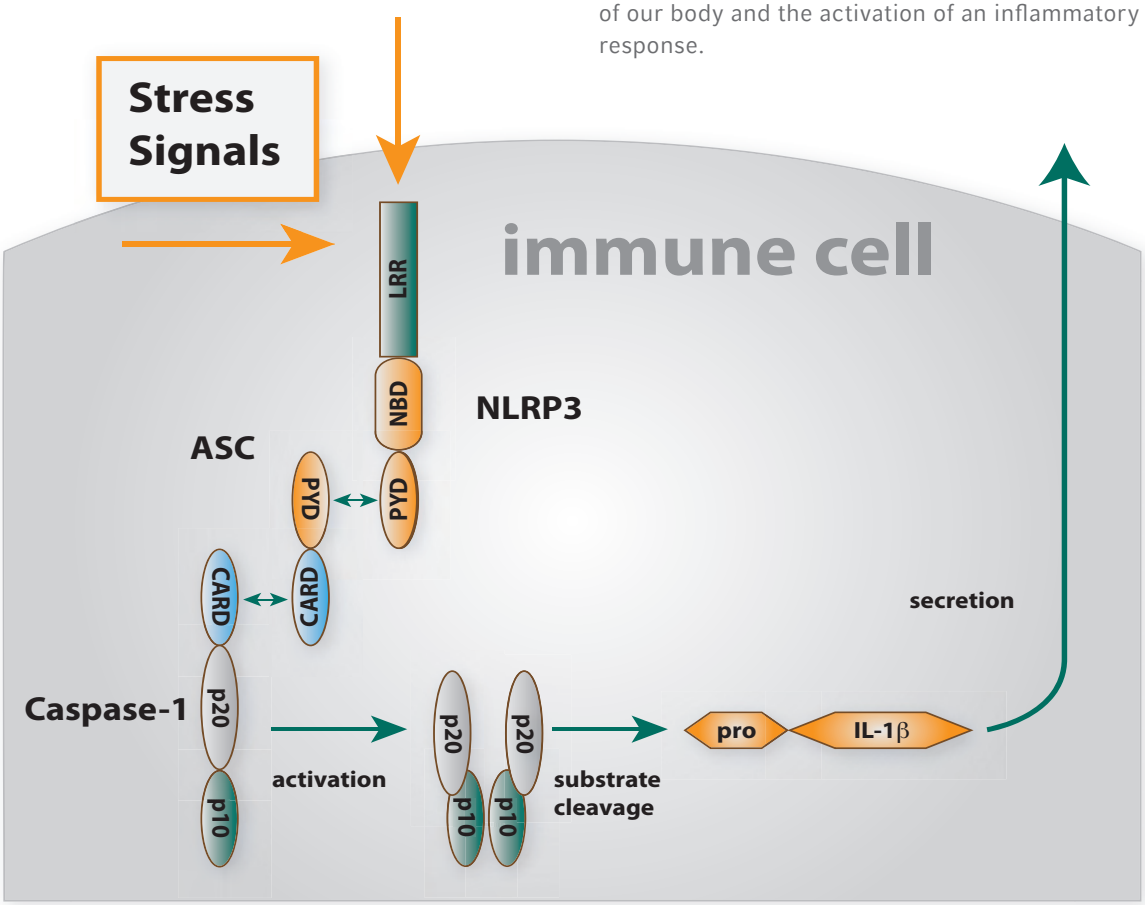

Figure 1: The cytoplasmic sensor protein NLRP3 responds to a combination of cellular stress signals, leading to the assembly of a protein complex consisting of NLRP3, ASC, and caspase-1. This complex, termed the inflammasome, promotes the activation of the protease caspase-1, and active caspase-1 cleaves the biologically inactive pro-form of IL-1β to generate the active cytokine. Active IL-1β is then secreted from the cell.

Goals of the project

► It is currently unknown how inflammasomes can control secretion of IL-1 and pyroptosis. Proteases are proteins that cut other proteins. The most prominent substrate of caspase-1 is the pro-form of IL-1β. This form is not secreted, and is also not capable of activating its receptor IL-1R1, since the pro-domain interferes with receptor binding. Secretion of IL-1β is observed in concert with cleavage, which has encouraged the view that these two processes are mechanistically connected. In our own recently published work, we demonstrated that this long-standing paradigm is not correct (Gross et al., Immunity, 2012). We could show that the ability of caspase-1 to regulate secretion of IL-1β is in fact independent of its protease activity. We found that caspase-1 forms stable complexes with currently unknown interaction partners specifically upon inflammasome activation, and hypothesize that these mediate the downstream effects of inflammasome activity. Furthermore, inflammasomes can control release of non-substrate proteins including IL-1α and potentially others that can contribute to the local and systemic effects of inflammasome activity.

We are using the powerful tools of proteomics to identify and characterize factors that associate with caspase-1 in an inflammasome-dependent manner in order to elucidate the mechanism by which caspase-1 controls IL-1 secretion and pyroptosis. Furthermore, we study the full repertoire of factors that are secreted in a caspase-1-dependent manner.

Future prospects and economic impact

► Dysregulated IL-1 production has a causal role in a number of acquired and hereditary auto-inflammatory conditions. These include particle-induced sterile inflammation (as is seen in gout, silicosis, and asbestosis), hereditary periodic fever syndromes, and metabolic diseases such as diabetes and atherosclerosis. In many cases, the pathogenesis of these conditions has been linked to deregulated inflammasome activation. Blocking the interaction of IL-1α and IL-1β with their common receptor, IL-1R1 is effective in conditions such as gout and hereditary periodic fever syndromes, underlining the critical role of IL-1 in these diseases. This is either done by increasing the extracellular levels of the natural IL-1 receptor antagonist through depletion of IL-1 using, for example, antibodies. This type of medication is not orally available but has to be given by frequent injections and is particularly costly. It may therefore be favourable to interfere with the process of IL-1 production by blocking inflammasome activation or IL-1 secretion.

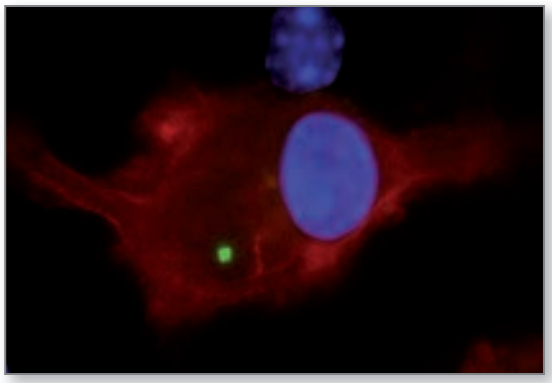

Figure 2: *Inflammasomes (green) form large, insoluble complexes upon activation. Dendritic cell; blue, nucleus; red, actin cytoskeleton.*

Conclusions and outlook

► The process of inflammasome activation and subsequent secretion of IL-1 secretion represents a major uncharted area of cell biology. We hope that a better understanding of these processes and the factors involved will unveil mechanisms that may serve as targets for future therapies.

Selected Publications

1. Gross O, Yazdi AS, Thomas CJ, Masin M, Heinz LX, Guarda G, Quadroni M, Drexler SK, and Tschopp J: „Inflammasome Activators Induce Interleukin-1a Secretion via Distinct Pathways with Differential Requirement for the Protease Function of Caspase-1." Immunity. 2012; 36(3): p. 388-400.
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3. Poeck H, Bscheider M, Gross O, Finger K, Roth S, Rebsamen M, Hannesschlager N, Schlee M, Rothenfusser S, Barchet W, Kato H, Akira S, Inoue S, Endres S, Peschel C, Hartmann G, Hornung V, Ruland J: „Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production." Nat Immunol. 2010; 11(1): 63-69.
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Bringing Order to Protein Disorder

We use structural biology to elucidate the intricate link between the function of disordered proteins, their regulation and human diseases

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Introduction

► "Function requires structure" – this is a common statement throughout structural biology. Indeed, most proteins need to adopt a defined three-dimensional structure to carry out their function. However, a large fraction of the genome of any organism encodes proteins that, completely or in part, do not adopt a defined three-dimensional structure but are nevertheless essential for cellular function: the so-called intrinsically disordered proteins (IDPs). Given their frequent occurrence in disease, it is of no doubt that these proteins are potential targets for drug development. To understand these proteins in general and to provide strategies how to modulate these interactions for disease treatment, structural characterization of IDPs and their biological complexes is the key.

We focus on the IDPs in the intersection of the Wnt and insulin signaling pathways which regulate cell fate, and contribute to diseases and ageing. Recent work has shown that regulation of these proteins involves an intricate 'code' of numerous post-translational modifications (PTMs) and binding of a plethora of co-factors. Deregulation of these proteins, including missense mutations and changes in the signaling state, leads to cancer, diabetes and ageing. Despite their importance, the underlying molecular mechanisms are still largely unknown. This is mainly due to their intrinsic flexibility, which complicates structural analysis by conventional approaches.

Goals of the project

► The central goal of this project is to identify the structural requirements of binding between structured proteins (i.e. ß-catenin) and the intrinsically disordered regions of Axin-1, APC, LEF-1 (Wnt signaling) and FOXO4 (insulin signaling). Given the importance of these proteins in human diseases, our results will contribute essential structural and functional data for understanding disease. These IDPs and their complexes are highly dynamic and challenging when relying on conventional techniques and need to be studied under near-native conditions. Therefore we apply our recently developed multidisciplinary approach in which Nuclear Magnetic Resonance (NMR) spectroscopy, Small-angle X-ray/neutron scattering (SAXS/SANS) and modeling strategies are combined to characterize the structural properties of these disease-related IDPs in isolation and in complex with their folded protein co-factors, the modulation of structure and interactions by modifications (PTMs, mutation), and the interference with small-molecule modulators with function in vitro and in vivo.

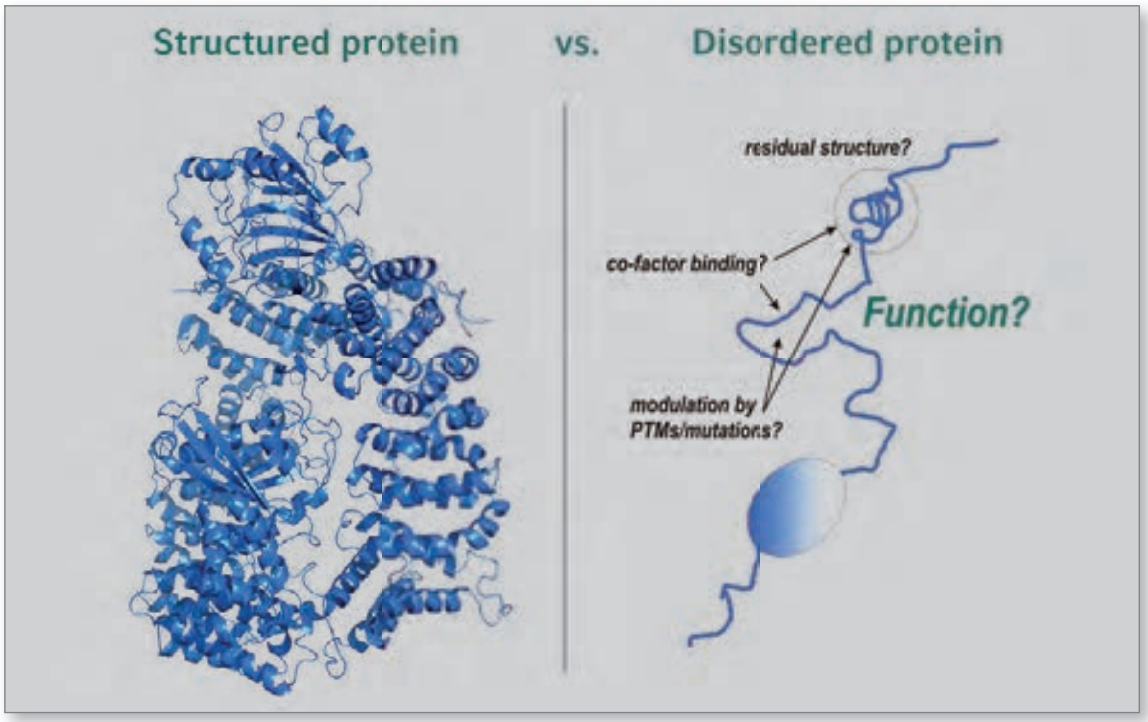

Figure 1: *Comparison of ordered and disordered proteins. We aim to characterize the structural properties of disordered proteins in isolation and in complex with their folded protein co-factors, the modulation of structure and interactions by modifications (PTMs, mutation), and the interference with small-molecule modulators with function.*

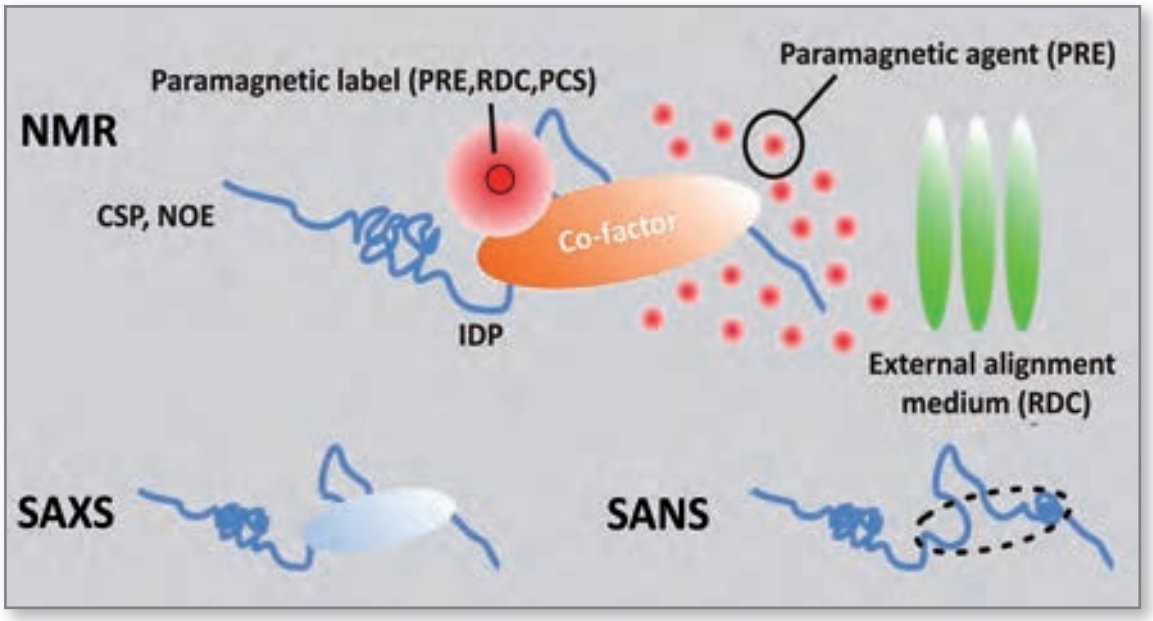

Figure 2: Outline of the multi-disciplinary approach for structure determination of dynamic protein complexes in solution by combining NMR spectroscopy, SAXS/SANS and modelling. CSP, chemical shift perturbation; NOE, nuclear Overhauser enhancement; PRE, paramagnetic relaxation enhancement; sPRE, solvent PRE; PCS, pseudo contact shift; RDC, residual dipolar coupling.

Cooperation partners

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Grenoble, France

Future prospects and general impact

► The molecular mechanisms of how IDPs act as binding platforms for many partners are far from being understood and therefore these proteins were mostly neglected as drug target. However, IDPs are known to bind with high specificity and therefore constitute a promising target for disease treatment. We anticipate that our results will set the base for a new generation of biomarkers for early detection, personalized anti-cancer and regenerative drugs targeting defects of the Wnt and insulin signaling pathways imposed by disease mutations or changes in the signaling state.

Conclusions and outlook

► In summary, we combine a cutting edge multi-disciplinary structure determination approach with a highly relevant biology of IDPs from the Wnt and insulin signaling pathways to allow efficient translation from in vitro structural and interaction data to in vivo biology and vice-versa. This holds the key to understanding these proteins in general and provides essential inside how to modulate these interactions for disease treatment.

Selected Publications

1. Dormann D., Madl T., Valori C.F., Bentmann E., Tahirovic S., Abou-Ajram C., Kremmer S., Ansorge O., Mackenzie I.R.A., Neumann M., Haass C., Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS, EMBO Journal (2012) accepted
2. Madl T., Güttler T., Görlich D., Sattler M., Structural Analysis of Large Protein Complexes using Solvent Paramagnetic Relaxation Enhancements, Angewandte Chemie 50 (2011), 3993-3997
3. Madl T., Gabel F., Sattler M., NMR and Small Angle Scattering-based structural analysis of protein complexes in solution, Journal of Structural Biology 173 (2011), 472-482
4. Madl T., Felli I.C., Bertini I., Sattler M., Structural analysis of protein interfaces from 13C direct-detected paramagnetic relaxation enhancements, Journal of the American Chemical Society 132 (2010), 7285-7287
5. Madl T., Bermel W., Zangger K.: Use of Relaxation Enhancements in a Paramagnetic Environment for the Structure Determination of Proteins Using NMR Spectroscopy, Angewandte Chemie 48 (2009), 8259-8262

Translational Control in Eukaryotes

Regulation of eukaryotic translation initiation by upstream open reading frames

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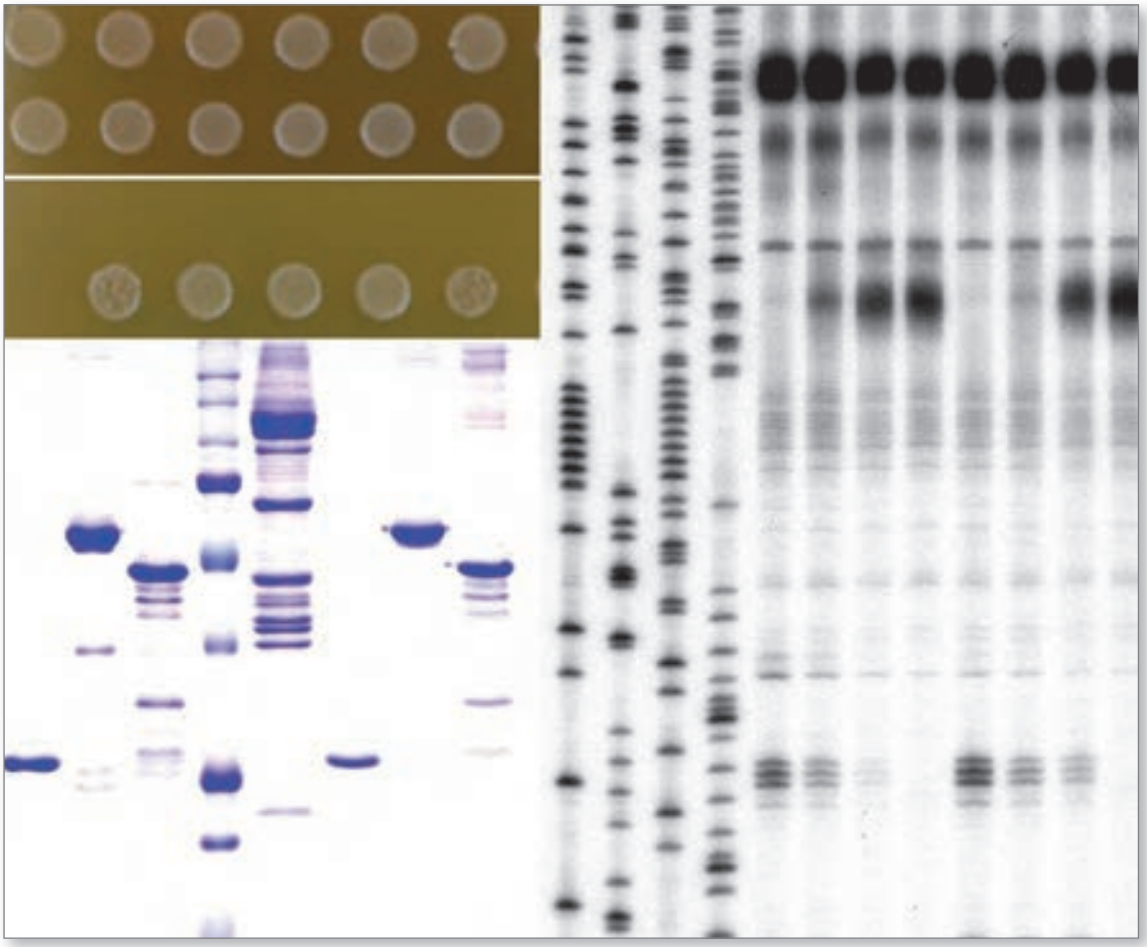
Introduction

► Our ability to sequence has far outpaced our understanding of the control of gene expression. With more and more genomes being sequenced it is now becoming increasingly important to understand in molecular detail how regulation is achieved. Even though we have a good knowledge of cellular RNA diversity and abundance this still does not allow us to infer from the transcriptome on the proteome. In fact one of the major challenges in modern biology is to bridge the evident gap between transcriptome and proteome which requires a better understanding of translational control.

Translation control by upstream open reading frames

► Translational control is an important and critical layer of gene expression control in essentially all eukaryotic cells. It allows both rapid and localized changes in the concentrations of proteins in responses to extra- and intracellular stimuli and it is crucial for a large number of important cellular processes such as cell division and differentiation, embryonic development and memory formation through neuronal plasticity.

Many mRNAs, particularly those encoding oncogenes, cytokines, growth factors etc., carry upstream open reading frames (uORFs) in their



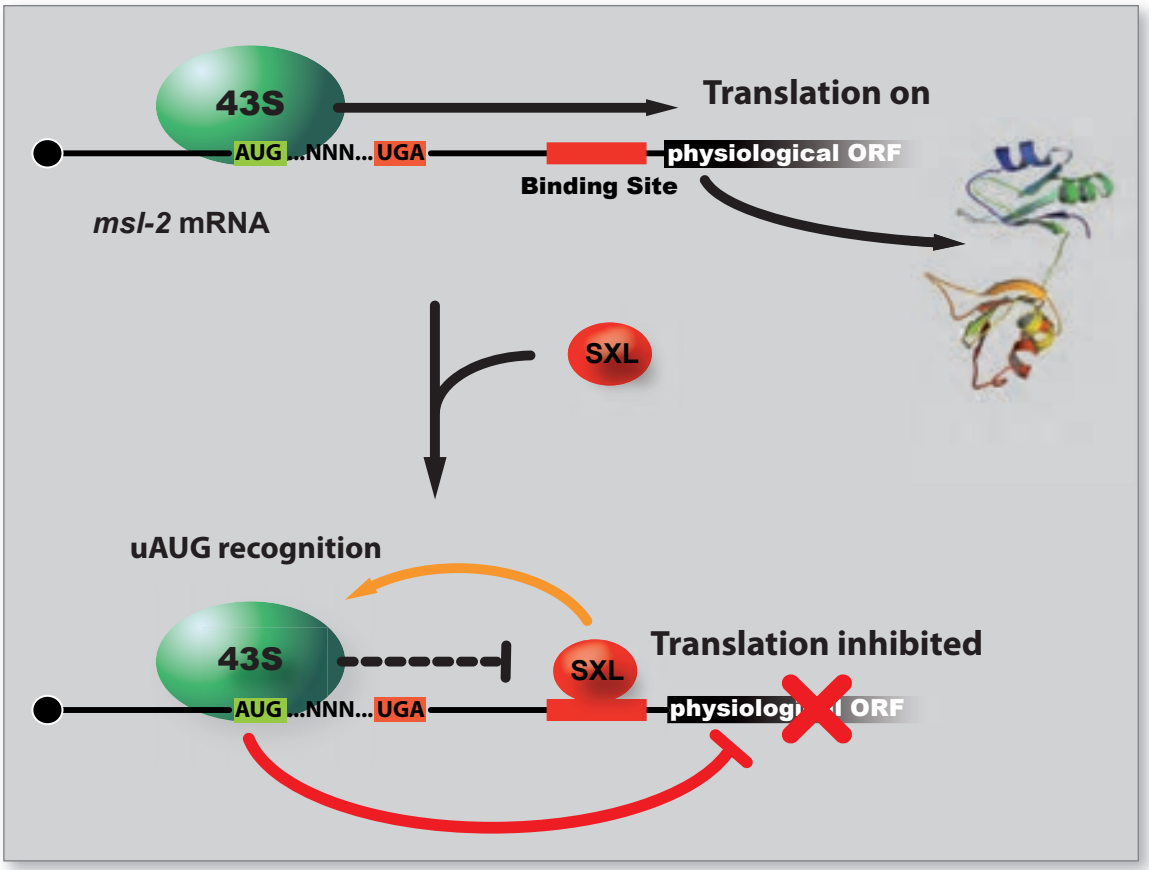


Figure 2: Translational control of msl-2 mRNA by the RNA-binding protein SXL: In the absence of regulatory RNA-binding protein the upstream open reading frame (uORF) in msl-2 mRNA only weakly impacts on downstream translation, resulting in translation of the physiological open reading frame (upper panel, the uORF initiation (AUG) and termination (UGA) codons are highlighted in green an red respectively). When recruited to an RNA regulatory element (orange box) in the 5' untranslated region of msl-2 mRNA, the RNA-binding protein SXL (orange sphere) is able to direct initiating ribosomes to the upstream initiation codon and augments the inhibitory potential of the uORF. This results in robust and strong translational repression of the msl-2 physiological open reading frame (lower panel).

5' untranslated regions (UTRs). These uORFs are widely recognized as constitutive regulatory elements that impinge on the translation of their associated physiological open reading frames, potentially affecting the protein output of half of the human transcripts. Polymorphisms that disrupt or create uORFs in human patients can result in or increase susceptibility to severe disorders such as hereditary thrombocythaemia, cancer, bipolar affective disorders, and Alzheimer's disease, underscoring the broad significance of the uORF regulatory potential.

Recently I could uncover a novel paradigm for the regulation of translation by demonstrating that a trans-acting factor – an RNA-binding protein – can modulate the activity of a uORF. Probing the generality of the underlying mechanism unraveled a network of co-regulated, natural *Drosophila* mRNAs revealing a novel and possibly systematic principle for the regulation of protein synthesis.

Future prospects and general impact

► Building on these findings I now plan to make use of a systematic and unbiased approach that aims to identify novel cases of protein-controlled uORFs, characterize their trans-acting regulatory RNA-binding proteins and study the regulatory mechanism in molecular detail, aiming for a thorough understanding of uORF-mediated translational control and its implication in disease.

Selected Publications

Medenbach, J., Seiler, M., and Hentze, MW.: Translational control via protein-regulated upstream open reading frames. Cell, 2011, 145(6):902-13.

Cooperation partners

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RNA: The Missing Link in Bacterial Pathogen-Host Interactions

Unraveling hidden roles of RNA in the interplay between bacteria and mammalian hosts

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Introduction

► Although the vast majority of bacteria are innocuous or even beneficial to mammalian hosts, pathogenic bacteria are a major threat to human health, being a leading cause of mortality and morbidity worldwide. Pathogenic bacteria contribute to important diseases, such as tuberculosis as caused by *Mycobacterium tuberculosis*, or the foodborne illnesses caused by *Listeria, Shigella* or *Salmonella* species. During the course of infection, bacterial pathogens manipulate a vast range of host cellular functions to ensure their survival and replication. Among others, bacterial pathogens are known to induce reorganization of the host cell cytoskeleton, modulate signal transduction pathways, membrane trafficking and pro-inflammatory responses. However, a largely unexplored question in the interplay between host and pathogens is the impact of bacterial infection on host cell RNA metabolism and its consequences on the bacterial life cycle. A proper RNA metabolism is essential to a number of crucial host cell functions and therefore it is not surprising that pathogens have evolved sophisticated mechanisms to subvert these pathways to their own benefit.

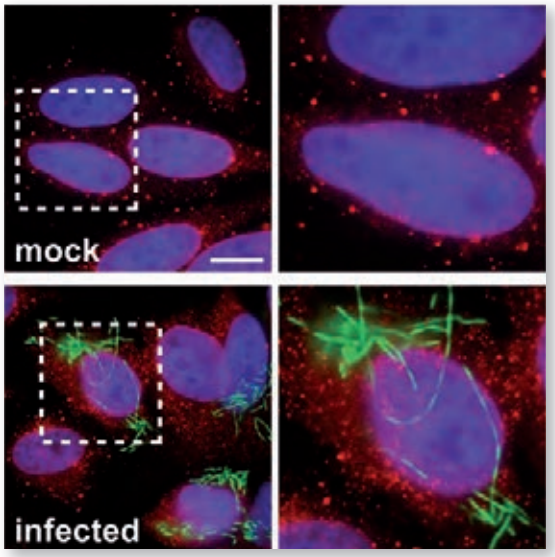

Figure 1: *Salmonella infection induces disassembly of cytoplasmic RNA processing bodies (P-bodies).*

Goals of the project

► MicroRNAs are a class of genome-encoded small RNAs that post-transcriptionally modulate the expression of cellular mRNAs, regulating a wide-range of biological processes. Previous studies have shown that bacterial pathogens induce significant changes in the mammalian host microRNA expression profile. For example, our previous work showed that a small subset of miRNAs, including the let-7 family that controls cytokine expression, is regulated in response to Salmonella infection. Nonetheless, there is currently no comprehensive atlas of microRNAs regulated as a consequence of bacterial infection.

A major focus of our research is the identification of host microRNAs that are regulated upon infection with several representative bacterial pathogens, as well as the characterization of the mechanisms by which bacteria modulate host cell microRNA expression. Our ultimate goal is to determine whether different bacteria regulate a common set of host microRNAs or if a microRNA signature exists for infection by different bacteria, and to analyze the cell-type specificity of this response. We are also working on the identification of host cell microRNAs that regulate bacterial infection. The identification of these microRNAs will be instrumental to the development of novel therapeutic approaches against pathogenic bacteria, either through the modulation of selected microRNAs, or their targets.

The relationship between bacterial infection and RNA granules, in particular P-bodies and stress-granules, is another aspect of the bacterial-host interaction for which very little information is available. Considering that the formation and stability of RNA granules is strictly dependent on the cellular RNA metabolism, any perturbation of these structures induced by bacterial pathogens will likely be linked to an impact on host cell RNA metabolism. The reciprocal effect of RNA metabolism on bacterial infection will also be evaluated. The overall aim of these studies is to characterize the impact of bacteria on cellular mRNA processing, stability and surveillance pathways.

Finally, we will address the long-standing question of whether or not RNA serves as a communication molecule during bacterial infection, such that bacterial small non-coding RNAs are transferred into host mammalian cells to modulate their function.

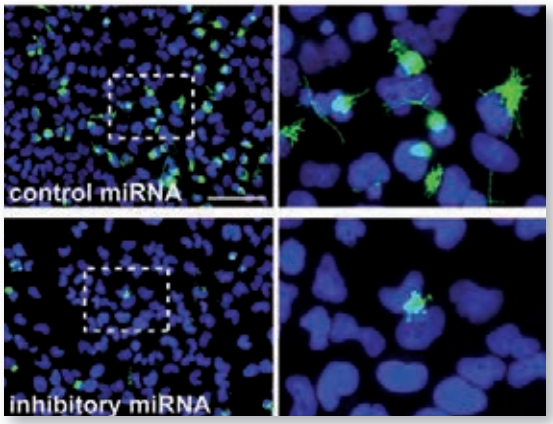

Figure 2: Overexpression of selected microRNAs inhibits Salmonella infection.

Conclusions and outlook

► Overall, by determining how bacterial pathogens interfere with the RNA metabolism of host mammalian cells, and whether we can manipulate RNA related pathways to antagonize bacterial infection, we will significantly increase the current understanding of host-pathogen interactions. The ensuing knowledge may also constitute the basis for the development of novel therapeutic approaches against infection by bacterial pathogens.

Selected publications

1. Eulalio A, Mano M, Dal Ferro M, Zentilin L, Sinagra G, Zacchigna S, Giacca M (2012) Functional screening identifies microRNAs inducing cardiac regeneration. Nature 492(7429):376-381
2. Eulalio A, Schulte L, Vogel J (2012) The mammalian microRNA response to bacterial infections. RNA Biol 9(6):742-750
3. Schulte L, Eulalio A, Mollenkopf H, Reinhardt R and Vogel J (2011) Analysis of the host microRNA response to Salmonella uncovers the control of major cytokines by the let-7 family. EMBO J 30(10):1977-1989
4. Eulalio A, Fröhlich K, Mano M, Giacca M and Vogel J (2011) A candidate approach implicates the secreted Salmonella effector protein SpvB in P-body disassembly. PLoS One 6(3):e17296
5. Eulalio A, Huntzinger E, Izaurralde E (2008) Argonaute and GW182 interaction is essential both for miRNA-mediated translational repression and mRNA decay. Nat Struct Mol Biol 15:346-353
6. Eulalio A, Huntzinger E, Izaurralde E (2008) Getting to the root of miRNA-mediated gene silencing. Cell132:9–14

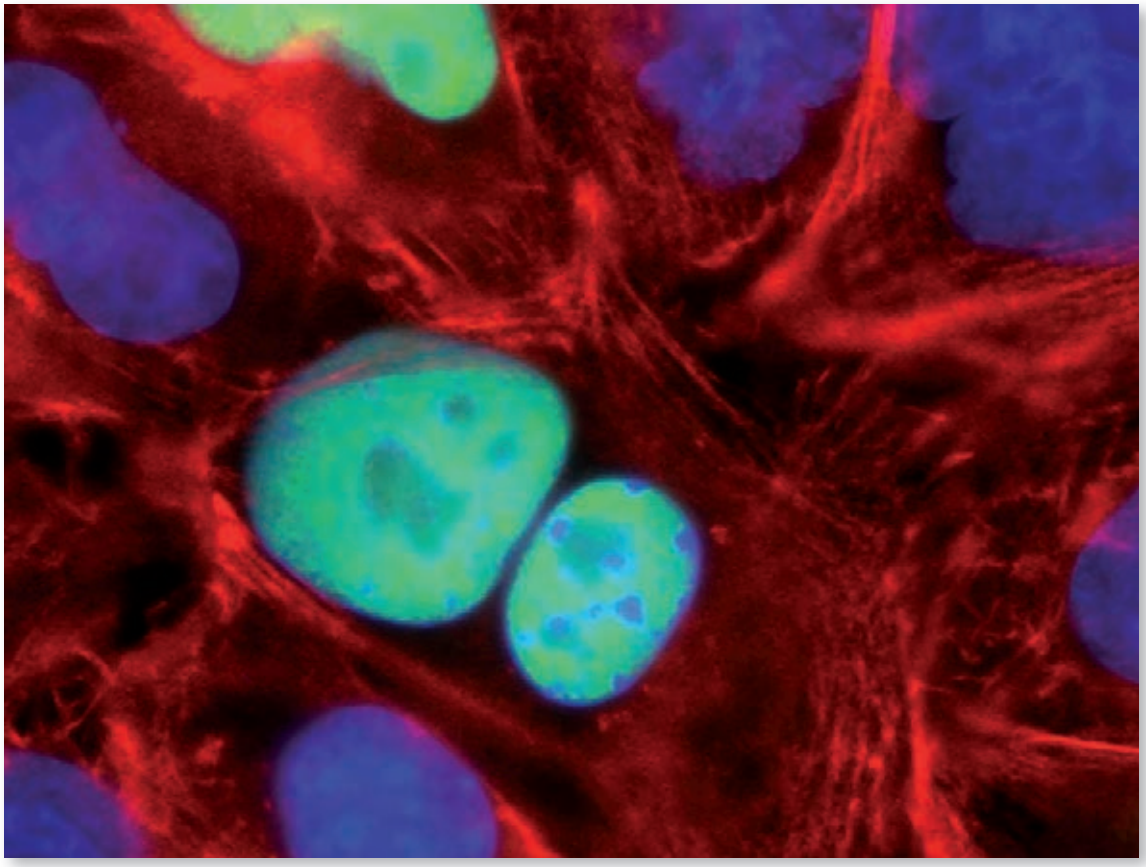

Figure 3: Analysis of the nucleo-cytoplasmic shuttling of a Salmonella effector protein using the heterokaryon assay.

Cooperation partners

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Transcriptome Analysis to Delineate Genes Involved in Synaptic Dysfunction in Synucleinopathies

Understanding mechanisms of neurite degeneration in Parkinson's disease and related disorders

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Cooperation partners

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Introduction

►Alpha-synuclein is a major constituent of Lewy bodies, and these protein aggregates are the pathological hallmark of Parkinson`s Disease (PD). While protein aggregates are the end stage of the disease, the early steps of alpha-synuclein toxicity are not clear.

Goals of the project

► We focus on the impaired cross talk between neurons at an early step of PD and investigate which additional genetic factors are involved in alpha-synuclein induced loss of connectivity between nerve cells and result in neuron dysfunction. Specifically, we will (1) Investigate alpha-synuclein induced gene expression signatures. We will therefore perform whole-transcriptome analyses in neuronal cultures from mouse models of synucleinopathies, which will give a specific chance to investigate the expression of a broad spectrum of individual synaptic proteins as well as to identify active biological processes and pathways within identified transcriptional profiles. Our preliminary data suggest spine loss in synucleinopathies, therefore (2) in a second step we will investigate potential pathways of spine disturbance in synucleinopathies in primary hippocampal cultures. In the next step we will analyze whether synaptic protein alterations are also present in PD patient derived neurons.

Conclusions and outlook

► Our data will contribute to a better understanding of synaptic connectivity in synucleinopathies and by transferring knowledge from murine models to human in vitro models delineate the cascade of neuronal dysfunction in PD and LBD. Defining postsynaptic genes involved in synucleinopathies may provide potential targets for therapeutic approaches therein.

Selected Publications

1. Winner B, Jappelli R, Maji SK, Desplats P, Boyer L, Aigner S, Hetzer C, Loher T, Vilar M, Campioni S, Tzitzilonis C, Soragni A, Jessberger S, Mira H, Consiglio A, Pham E, Masliah E, Gage FH and Riek R. In vivo demonstration that a-synuclein oligomers are toxic. PNAS. 108(10):4194-9.
2. Winner B, Melrose HL, Zhao C, Hinkle KM, Yue M, Kent C, Braithwaite AT, Ogholikhan S, Aigner R, Winkler J, Farrer MJ, Gage FH. Adult neurogenesis and neurite outgrowth are impaired in LRRK2 G2019S mice. Neurobiol Dis. 2011, 41(3):706-16.
3. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, Gage FH, Glass CK. A Nurr1/CoREST transrepression pathway antagonizes neurotoxicity of activated microglia, Cell. 2009; 137: 47-59.

Coworker

Dr. Francesc Perez-Branguli

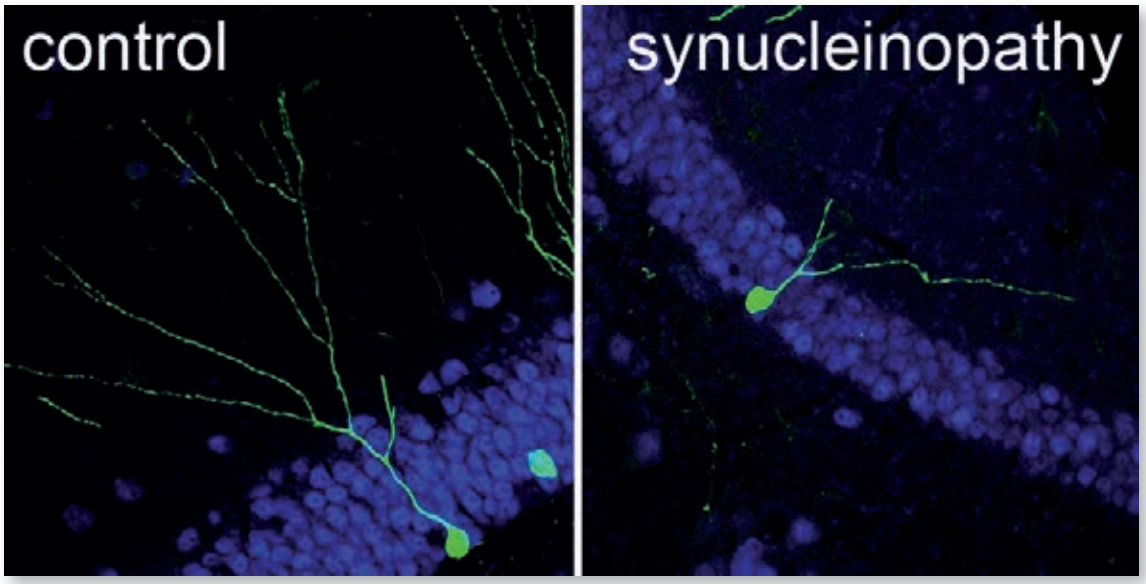

Figure 1: *Comparison of neurites in newly generated neurons in the dentate gyrus of the hippocampus. Neurites in synucleinopathies (labeled green) are shorter than controls.*

Physical Analysis of Nucleosome Positioning, Remodeling, and Transcription Regulation in Yeast

Reverse-engineering molecular interactions and mechanisms using coarse-grained physical models

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Introduction

► A central goal of molecular systems biology is to unravel the mechanisms of eukaryotic transcription regulation, for which yeasts provide the simplest model systems. Since direct observation of these mechanisms is not feasible, they must be reconstructed from measurable characteristics such as nucleosome positions, binding of transcription factors, and transcription rates. For this reconstruction process, theoretical models are an essential tool. They can integrate multiple binding interactions and active enzymatic mechanisms into a quantitative description of the system behavior. The quantitative description then permits to test whether mechanistic hypotheses are compatible with the data.

Goals of the project

► Our goal is to apply this general approach in the context of nucleosome positioning, in close collaboration with the experimental group of Dr. Philipp Korber (LMU). The positions of nucleosomes on a eukaryotic genome determine which parts of the DNA are readily accessible, e.g. for binding of transcription factors (TFs), and conversely, the binding of TFs also affects the positioning of nucleosomes. DNA accessibility is modulated by various chromatin remodeling complexes and histone modification enzymes, many of which are specifically recruited by TFs. The central goal of this project is to construct coarse-grained physical models to disentangle various effective interactions that determine nucleosome positions in vivo and also in vitro.

Future prospects and general impact

► Such a physics-based description will contribute towards the larger scale effort in biology to unravel the interplay of transcription factors, the core transcription machinery, and chromatin, that ultimately results in the complexity of cis-regulatory gene regulation.

Selected Publications

1. N. Geisel and U. Gerland (2011)
   Physical limits on cooperative protein-DNA binding and the kinetics of combinatorial transcription regulation,
   Biophys. J. 101, 1569–79.
2. W. Möbius and U. Gerland (2010)
   Quantitative test of the barrier nucleosome model for statistical positioning of nucleosomes up- and downstream of transcription start sites.
   PLoS Comp. Biol. 6, e1000891.
3. W. Möbius, R. Neher, and U. Gerland (2006)
   Kinetic accessibility of buried DNA sites in nucleosomes.
   Phys. Rev. Lett. 97, 208102.

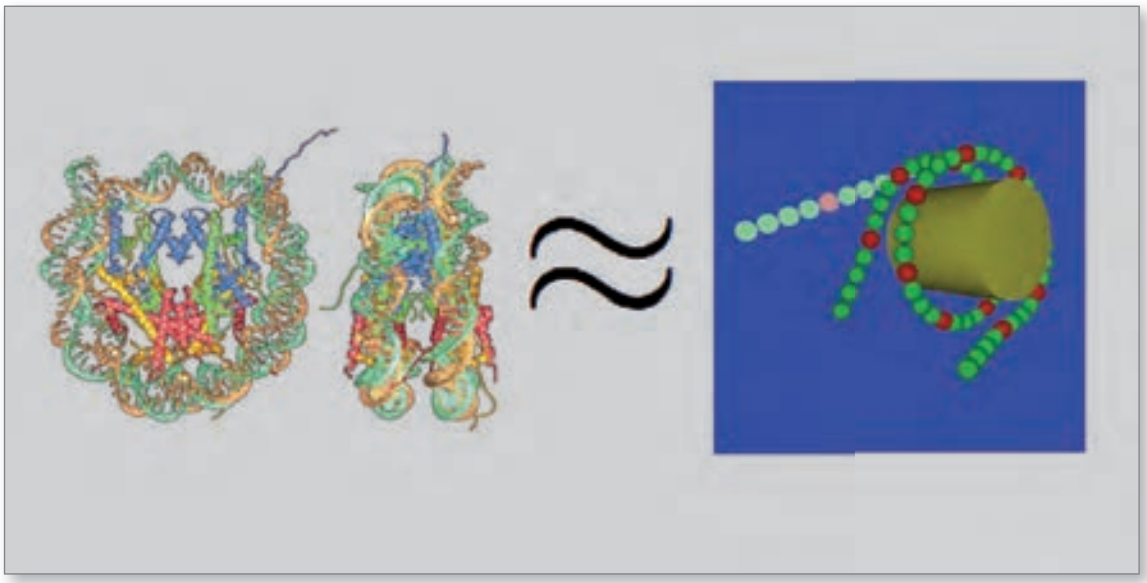

Figure 1: *Illustration of a coarse-grained physical model for a nucleosome. It approximates the highly dynamical biomolecular complex by a physical object with relatively few dynamical degrees of freedom. Clearly, the challenge is to find reduced descriptions which retain the degrees of freedom that are essential for the biological context at hand.*

Small RNA Mediated Heterochromatin Formation in Fission Yeast

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Introduction

► Regulation of genome expression is essential for cell proliferation, differentiation, development and viability. Small RNA silencing pathways are involved in the cellular control of gene expression and in the protection of the genome against mobile repetitive DNA sequences, retroelements and transposons. Small RNAs interact with target RNAs and promote chromatin modifications, translational inhibition or degradation of complementary RNAs. We have uncovered new class of small RNAs in fission yeast, priRNAs, which are generated independently of Dicer.

Goals of the project

► priRNAs are likely to be involved in triggering of siRNA amplification and heterochromatin assembly within centromeric repeats. However, it remains unclear how priRNAs are generated. We will combine small RNA sequencing and transcriptomic approaches with biochemistry and functional assays to determine biogenesis and function of priRNAs. We also study why some RNAs are more susceptible to RNA interference and how RNAi is recruited to transposable and repetitive elements in fission yeast. To study RNAi recruitment to chromatin we will use cryo electron microscopy to determine structures of RNAi and chromatin complexes in fission yeast. Defects in small RNA-mediated regulation of genome expression have been described in several human cancers. Fundamental understanding of the role of small RNAs in gene regulation will help us understand why some cells lose their identity and turn into cancer cells.

Selected Publications

1. Halic M and Moazed D
Dicer-Independent priRNAs and Argonaute Trigger RNAi and Heterochromatin Formation Cell. 2010 Feb 19;140(4):504-516

2. Halic M, Blau M, Becker T, Mielke T, Pool MR, Wild K, Sinning I, Beckmann R Following the Signal Sequence from the Ribosomal Tunnel Exit to Signal Recognition Particle Nature. 2006 Nov 23;444(7118):507-11.

3. Halic M, Gartmann M, Schlenker O, Mielke T, Pool MR, Sinning I, Beckmann R Signal recognition particle receptor exposes the ribosomal translocon binding site.
Science. 2006 May 5;312(5774):745-7

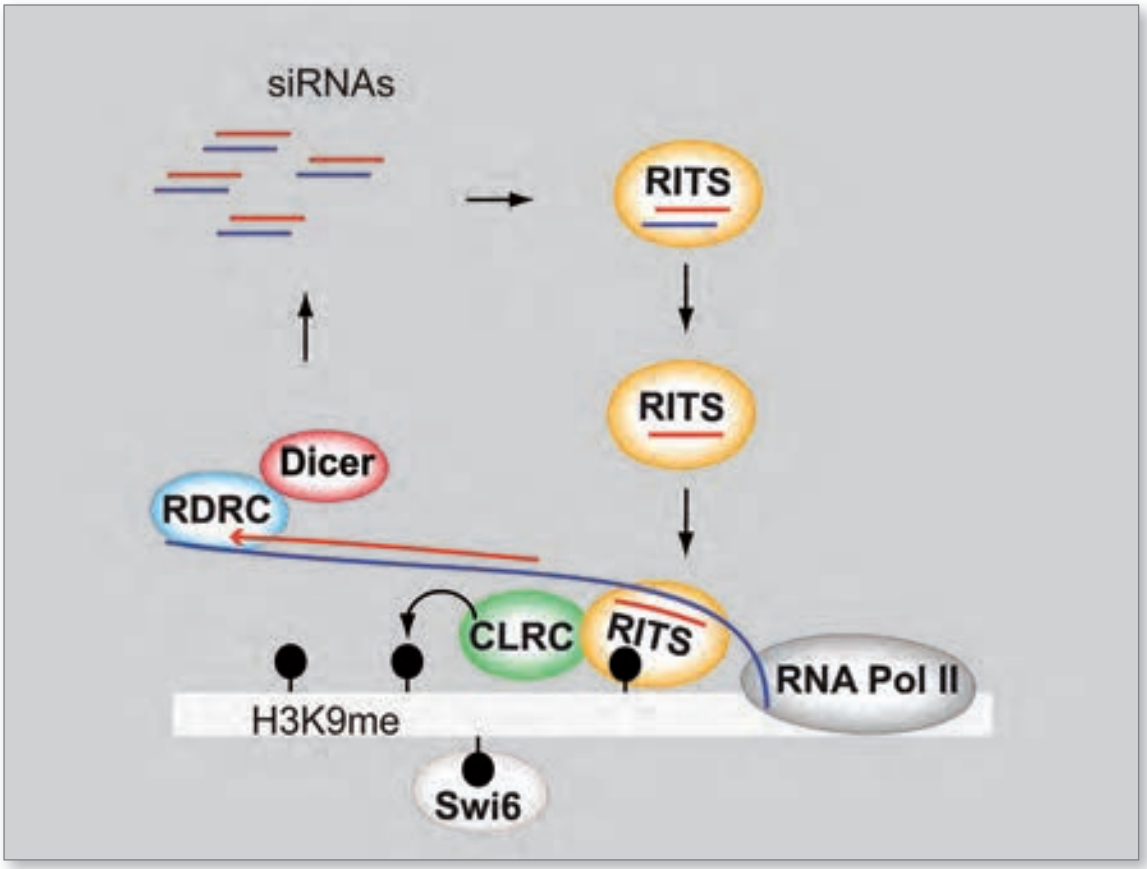

Figure 1: *siRNA mediated heterochromatin formation in fission yeast. RITS complex (Argonaute) recruits RDRC (RNA dependent RNA polymerase complex) and Dicer to synthesize new dsRNA and amplify siRNAs. RITS also recruits CLRC complex to modify chromatin structure with repressive H3K9 methylation. H3K9 methylation recruits HP1 protein Swi6 and induces heterochromatin formation.*

Reconstitution of Nucleosome Positioning Mechanisms for *S. cerevisiae* and *S. pombe*

Comparative *in vitro* genomics over a large evolutionary distance

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Cooperation partners

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Sweden

Prof. Dr. Craig L. Peterson
Program in Molecular
Medicine, University of
Massachusetts, Medical
School Worcester,
USA

Introduction

► The genetic information of all cells is encoded in DNA. However, in eukaryotic cells the DNA is not just a "naked fibre" curled up in the nucleus, but it is intricately packaged into a protein-nucleic acid-structure called chromatin. The basic packaging units of chromatin are so called nucleosomes. Each nucleosome holds 147 bp of DNA and most of the genome is organized this way. Importantly, nucleosomes impede access to DNA such that their non-random distribution along the DNA is a fundamental layer of gene regulation. In other words, the genetic information encoded in the DNA sequence is regulated by the epigenetic information encoded in chromatin. While this principle is true for eukaryotes in general, there are important mechanistic differences between species.

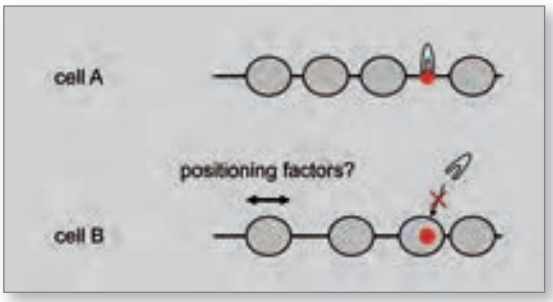

Figure 1: *Epigenetic information of nucleosome positioning. Nucleosome positions (grey circles) along DNA (line) may differ in cell A versus cell B, such that the binding site (red dot) for a protein factor (blue arc) may be accessible or not.*

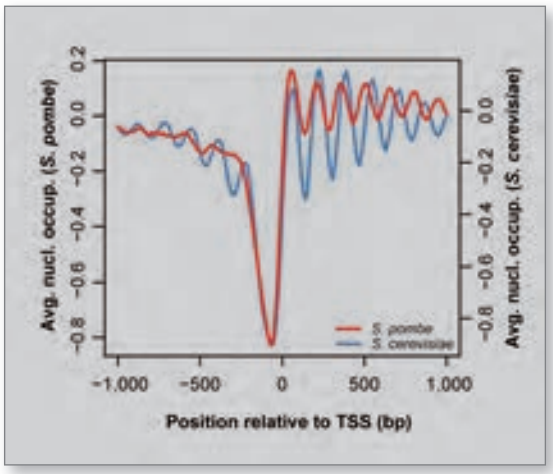

Figure 2: *Similarities and differences of global nucleosome positioning in S. cerevisiae and S. pombe. Both yeasts show a minimum of nucleosome occupancy just upstream of transcriptional start sites (TSS) and regular peak arrays downstream, but S. pombe has hardly any arrays upstream of TSS and shorter peak-to-peak distance (nucleosome spacing). Figure from Lantermann et al., 2010, Nat. Struct. Mol. Biol.*

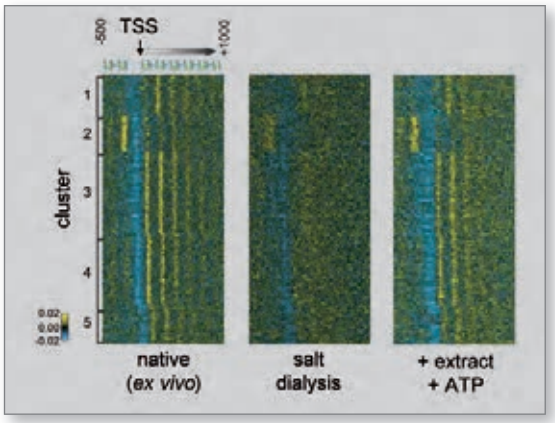

Figure 3: *Genome-wide in vitro reconstitution of in vivo-like nucleosome positions for S. cerevisiae. Stacks of >4700 rows (= genes) with colour coded (yellow for high, blue for low), TSS-aligned and clustered nucleosome occupancy data. Only some native nucleosome positions are reconstituted by salt dialysis (just DNA and nucleosomes), but added extract/ ATP generates in vivo-like nucleosome positions. Data from Zhang et al., 2011, Science.*

Goals of the project

► We wish to understand the molecular mechanisms for nucleosome positioning in a genome-wide *in vitro* reconstitution approach combined with theoretical modelling (collaboration with the group of Prof. Dr. U. Gerland). In order to distinguish universally conserved from species-specific features, we compare the evolutionarily very distant unicellular yeasts *S. cerevisiae* and *S. pombe*.

Conclusions and outlook

► Our approach is special as it combines "vertical" (biochemistry of *in vitro* reconstitution), "horizontal" (-omics) and theoretical (modelling) techniques. This has the potential of defining quantitatively the mechanisms for the most basic level of eukaryotic genome organization.

Selected Publications

1. Zhang, Z. ‡, Wippo, C.J. ‡, Wal, M., Ward, E., Korber, P.*, and Pugh, B.F.* (2011) A packing mechanism for nucleosome organization reconstituted across a eukaryotic genome. Science, 332, 977-980.
2. Wippo, C.J., Israel, L., Watanabe, S., Hochheimer, A., Peterson, C.L., and Korber, P. (2011) The RSC chromatin remodeling enzyme plays a unique role in directing the accurate positioning of nucleosomes. EMBO J., 30, 1277-1288.
3. Lantermann, A.B.‡, Straub, T.‡, Strålfors, A., Yuan, G.-C., Ekwall, K.*, and Korber, P.* (2010) *Schizosaccharomyces pombe* genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of *Saccharomyces cerevisiae*. Nat. Struct. Mol. Biol., 17, 251-257.

(* shared corresponding authors, ‡ shared first authors)

Analysis and Modeling of Regulatory Protein-DNA Binding Energy Landscapes

Physical models and software to precisely measure and predict the binding of activator and repressor proteins to sequences in the genome

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Introduction

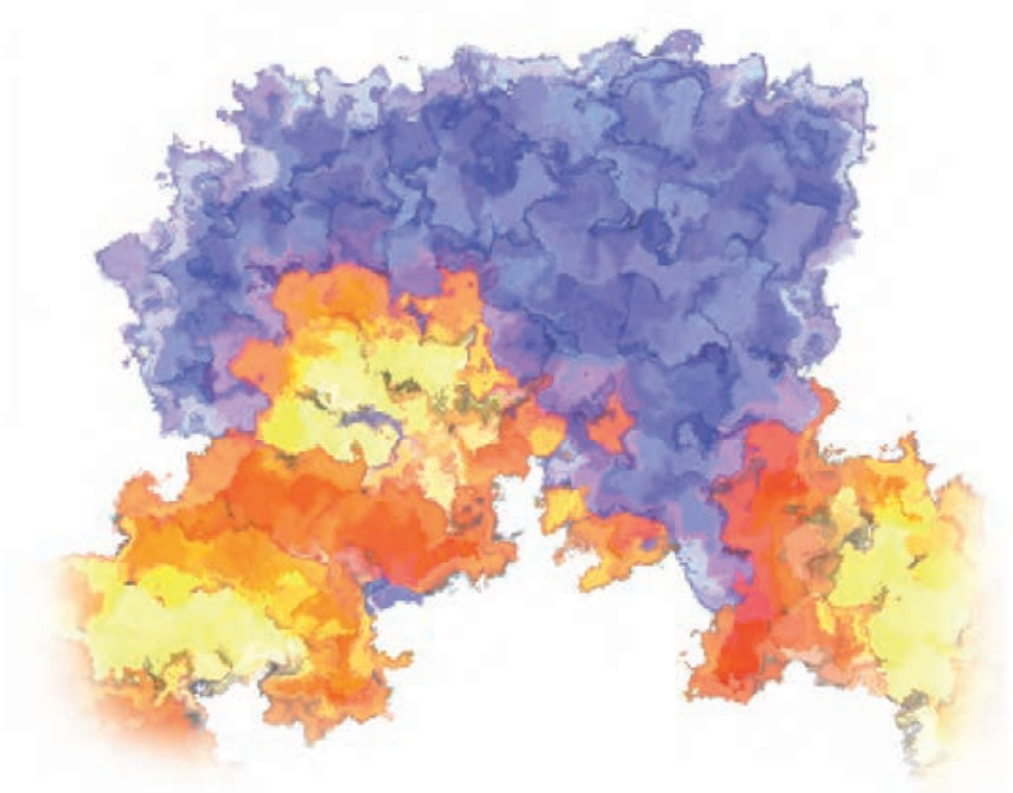
► The transcription rate of a gene is regulated by the coordinated binding of activating or repressing transcription factors at its promoter and cis-regulatory elements. To be able to understand the molecular computation which transforms factor concentrations into a transcriptional output, we need to know the precise binding affinities of the factor involved. Nutiu et al. (Nature Biotechnology 2011) have demonstrated a high-throughput method (high-throughput sequencing - flourescent ligand interaction profiling, or HiTS-FLIP) with which one can measure the binding constants of a protein factor to tens of millions of DNA sequences at a time in a single lane of a high-throughput sequencer. Using this method, charting the binding landscapes of thousands of factors in humans and model organisms seems within reach. This should allow the accurate prediction of regulatory network topologies, factor binding strengths and transcription rates.

Goals of the project

► In the framework of the BioSysNet network, we will develop theoretical methods and software to analyze and interpret these measurements and to derive models parameterizing the binding landscapes that allow for the dependence between motif positions. We further plan to use HiTS-FLIP to analyze the cooperative binding enery landscapes of interacting pairs of factors. Our work will contribute to a quantitative modeling of transcriptional regulation and to enabling a system-level description of regulatory networks in development and disease.

Selected Publications

1. Hartmann, H., Guthöhrlein, E. W., Siebert, M., Luehr, S., and Söding, J.; P-value based regulatory motif discovery using positional weight matrices. Genome Res., in press. doi: 10.1101/gr.139881.112 (2012).
2. Remmert M., Biegert A., Hauser A., and Söding J.; HHblits: Lightning-fast iterative protein sequence searching by HMM-HMM alignment. Nat Methods 9:173-175 (2011)
3. Mayer A*., Lidschreiber M*., Siebert M*., Leike K., Söding J., and Cramer P; Uniform transitions of the general RNA polymerase II transcription complex. Nat Struct Mol Biol. 17:1272-1278 (2010)
4. Biegert A. and Söding J.; Sequence context-specific profiles for homology searching. Proc Natl Acad Sci USA 106:3770-5 (2009)



$$E_{\text{bound}} = \sum_{i=1}^{l} E(i, X_i) + \sum_{i=n}^{l} E(i, X_i, X_{i+1}) + \ldots$$

How Stem is a Stem Cell?

Quantifying differentiation phenotypes using molecular transcription levels and single-cell genealogies of stem cell differentiation

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Introduction

► A central question in systems biology is how to link observed phenotypical behaviour to molecular state changes. We are specifically interested in the cellular phenotype and its change over differentiation in the context of somatic and embryonic stem cells (SCs) of mice. The challenge lies in identifying and quantifying regulatory mechanisms on the transcriptional level that cause a SC to differentiate into a particular cell type, potentially over many generations and dependent on its environment. Population heterogeneities as well as putative stochastic differentiation events require single-cell observations. In collaborations with our local SC institute, we have therefore imaged live hematopoietic and embryonic SCs and their progeny in vitro, and quantified transcription factor levels using fluorescence-tagged fusion proteins.

Goals of the project

► In this project, we ask which properties at which stage in a cellular genealogy are sufficient to predict differentiation. We determine morphological properties by quantifying the brightfield and phase-contrast movies, which up to now have only been used to track cells. We train a classifier separately on the phenotypical as well as the molecular data to see which features are sufficient to predict cell fate. Methodologically, we need to advance functional data classification and take into account dependence of samples from adjacent generations. In a first step, we evaluate our tools on a generative genealogy model. Eventually we will apply the predictors to existing and emerging differentiation trees to determine the time point of cellular decision on the single-cell level. The ability to predict cell decisions in real time will open up novel experimental approaches such as omics-analyses of cells as well as molecular perturbations at the time point of fate decision.

Conclusions and outlook

► On the translational side, we are aiming in the long run at improving the efficiency of differentiation protocols, thereby contributing to the perspective of stem cells in the treatment of severe diseases such as dementia or leukemia.

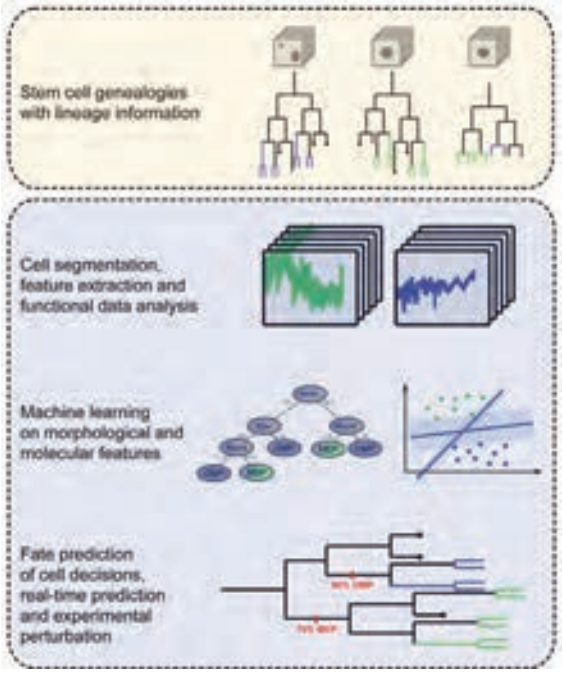

Figure 1: *From the stem cell genealogies provided by our collaboration partner, we extract morpholigical and molecular features. Using functional data analysis and machine learning, we will finally predict fate decisions in real-time to allow for targeted experimental perturbations.*

Selected Publications

1. Krumsiek, J., Marr, C., Schroeder, T., and Theis, F. J. Hierarchical differentiation of myeloid progenitors is encoded in the transcription factor network. PLoS ONE 6, 8 (2011), e22649.

2. Schwarzfischer, M., Marr, C., Krumsiek, J., Hoppe, P. S., Schroeder, T., and Theis, F. J. Efficient fluorescence image normalization for time lapse movies. Microscopic Image Analysis with Applications in Biology (2011).

3. Marr, C., Strasser, M., Schwarzfischer, M., Schroeder, T., and Theis, F. J. Multi-scale modeling of GMP differentiation based on single-cell genealogies. FEBS Journal (2012), 279, 18 (2012), 3289–3528.

Spatiotemporal Control of mRNA Levels

A molecular tool to study local protein synthesis

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Introduction

► Gene regulation is a logistic challenge for widely arborized neurons that need to follow local cues during their outgrowth, maintain regional homeostasis, and quickly respond to synaptic inputs at sites a great distance away from their cell body.

Goals of the project

► Local mRNA storage and protein synthesis have been shown to be important for axonal guidance, synaptogenesis, and synaptic plasticity consistent with hypotheses on quasi-autonomous local protein synthesis in neurons. It has however remained technically challenging to identify the kinetics and function of local mRNA translation and understand the parameters of its regulation.

Future prospects and general impact

► In this project, we thus seek to develop a more efficient genetic method for spatiotemporal regulation of specific mRNA levels. This will be pursued by a combination of protein engineering and molecular imaging.

Conclusions and outlook

► The technique aims at enabling systematic analyses of regulation and function of local protein expression and may find broader applications in tissue engineering and interventional studies.

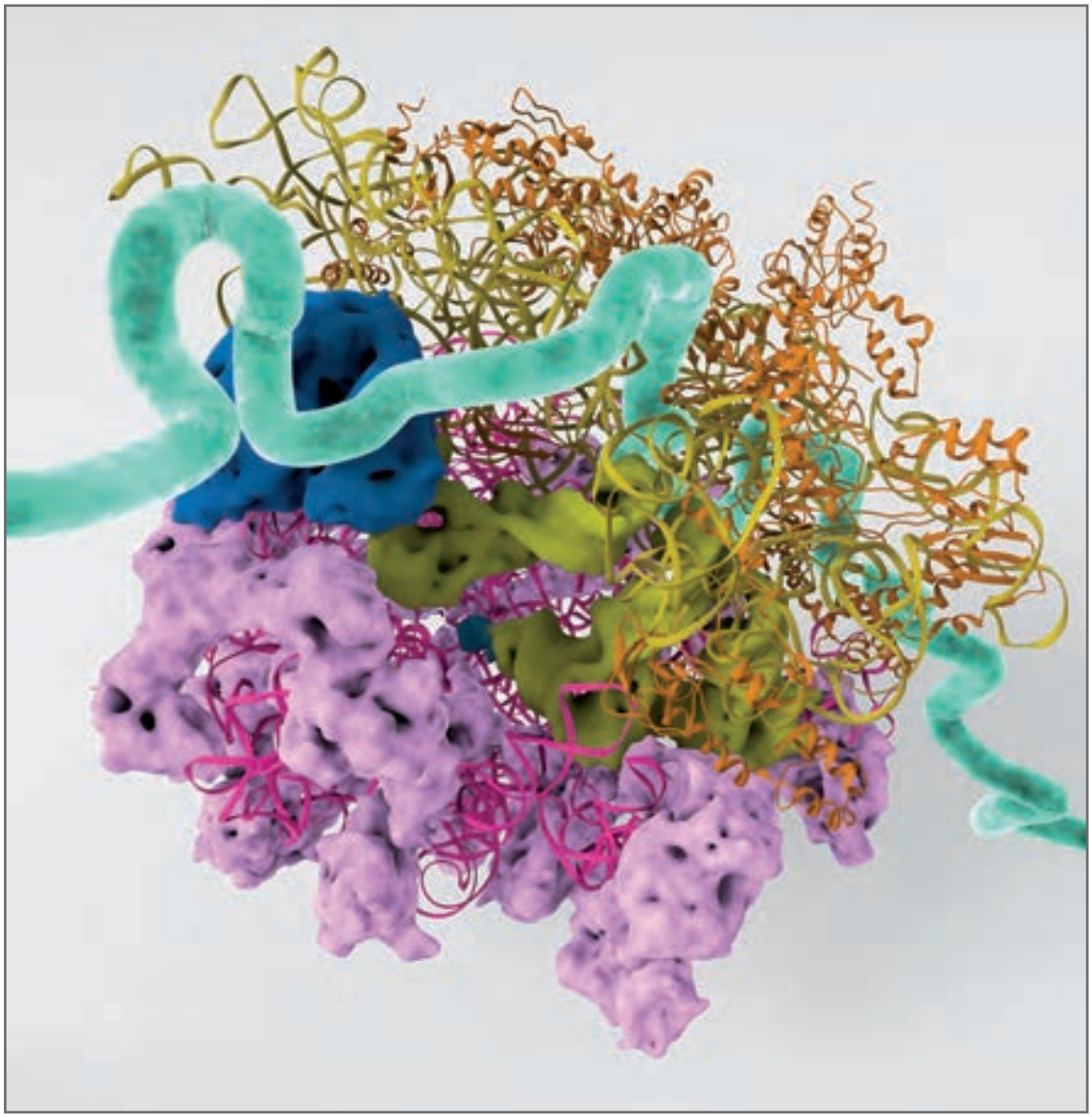

Figure 1: *Artistic depiction of a ribosome engaged in protein synthesis.*

Exploring RNA-binding Proteins in *Campylobacter jejuni*

Identification and functional characterization of RNA-protein complexes involved in post-transcriptional regulation in the human pathogen *Campylobacter jejuni*

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Introduction

► Post-transcriptional regulation is an important layer of gene-expression control in both pro- and eukaryotes. In bacteria, the 50 to 200-nt long small regulatory RNAs (sRNAs) constitute a major class of post-transcriptional regulators and have been implicated in bacterial stress response and virulence regulation. Besides sRNAs, a variety of RNA-binding proteins have crucial roles in post-transcriptional regulation and RNA metabolism in the cell. In many bacteria, the Sm-like protein Hfq is the most important RNA chaperone and is required for diverse cellular processes and virulence of many bacterial pathogens. Epsilonproteobacteria, including several important pathogens such as *Helicobacter pylori* and *Campylobacter jejuni,* do not carry an apparent Hfq homologue, and thus were previously thought to lack sRNA-mediated regulation (riboregulation). *Campylobacter* is currently the most common cause of bacterial gastroenteritis in humans and has also been associated with several autoimmune disorders. Its small genome (1.65 Mb) encodes only a few transcriptional regulators, indicating that also *Campylobacter* relies on post-transcriptional regulation and might use other RNA-binding proteins than Hfq to control its gene expression.

► Massively parallel cDNA sequencing (RNA-seq) has been revolutionizing transcriptome analyses of eukaryotes and prokaryotes. For example, our recently developed differential RNA sequencing approach (dRNA-seq) is selective for the analysis of primary transcriptomes (1). We have now applied this dRNA-seq approach to multiple *Campylobacter* strains, which allowed us to provide global maps of transcriptional start sites as well as conserved and strain-specific sRNA candidates. However, the functional roles and cellular targets of these sRNAs and their associated protein factors are still elusive.

Goals of the project

► In this project, we will use *Campylobacter* jejuni as a new model organism for riboregulation in bacteria without Hfq. We aim at the identification and functional characterization of protein factors involved in post-transcriptional regulation in *Campylobacter*. This will include a combination of genetic screens and the isolation of RNP-complexes, followed by proteomics and RNA-seq, as well as subsequent biochemical and structural analyses. For example, we will combine co-immunoprecipitation (co-IP) with RNA-seq to identify the RNAs bound to epitope-tagged RNA-binding proteins (2) and affinity chromatography of tagged sRNAs (3) to isolate RNA-protein (RNP) complexes (see Figure 1). The identification and characterization of novel RNP complexes in *Campylobacter* will increase our knowledge about gene regulation and also virulence control in a broader range of bacteria and help to understand the underlying mechanisms of post-transcriptional regulation in bacteria without Hfq. Moreover, the identification of novel virulence regulators will provide new targets for antimicrobial therapies.

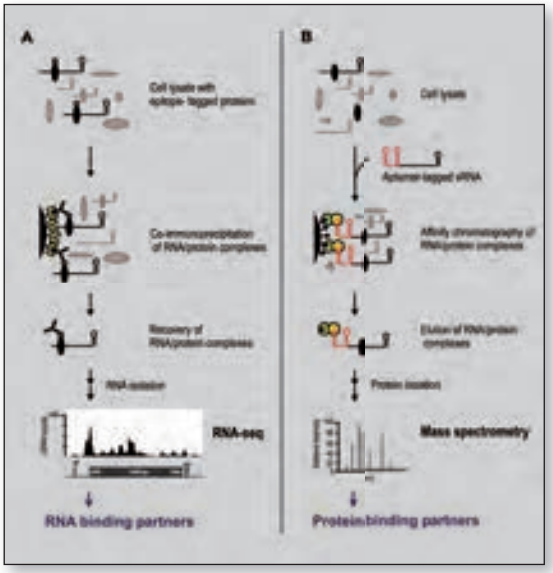

Figure 1: *Isolation of RNA/protein complexes using (A) co-IP of epitope-tagged proteins combined with RNA-seq and (B) affinity purification of aptamer-tagged sRNAs.*

Selected Publications

1. Sharma, C.M., Hoffmann, S., Darfeuille, F., Reignier, J., Findeiss, S., Sittka, A., Chabas, S., Reiche, K., Hackermüller, J., Reinhardt, R. et al. (2010) The primary transcriptome of the major human pathogen *Helicobacter pylori*. Nature, 464, 250-255.
2. Sittka, A., Lucchini, S., Papenfort, K., Sharma, C.M., Rolle, K., Binnewies, T.T., Hinton, J.C. and Vogel, J. (2008) Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. PLoS Genet, 4, e1000163.
3. Rieder, R., Reinhardt, R., Sharma, C.M. and Vogel, J. (2012) Experimental tools to identify RNA-protein interactions in *Helicobacter pylori*. RNA Biology, 9.

Transcriptional Priming as Global Mechanism Controlling Self Renewal and Differentiation during Hematopoietic Development

Examining transcriptional dynamics to study leukemia

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Introduction

► Leukemia is the consequence of a series of wrong decisions taken by a blood cell. A normally transient transcriptional program becomes permanent. Genes intended to ensure temporary expansion of progenitors now fuel the limitless expansion of malignant clones stimulated by runaway transcriptional elongation. Because this particular mode of transcriptional tuning is characteristic for many genes involved in developmental and differentiation decisions it can serve as a mark, flagging these important switchboards.

Goals of the project

► In this project we want to investigate the global landscape of transcriptional kinetics in normal and malignant cells. We are using conditional oncogenes to study the global changes accompanying the path from normal to leukemic stem cell and back. This will not only identify the important players but it will add another dimension by following these up in time. Like going from a snapshot to a movie we try to elucidate the kinetics of malignant transformation.

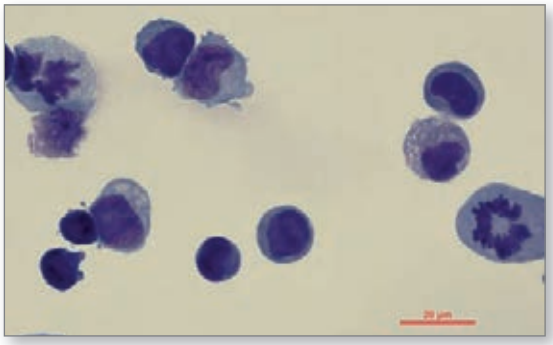

Figure 1: *Leukemia cells under the microscope.*

At the fluorescence activated cell sorter.

Future prospects and general impact

► The comprehensive understanding of the inner mechanics of leukemia development will show the Achilles' heel of this procedure. Only if one knows the "moving parts" it will be possible to point out therapeutically promising targets where blocking the sprocket will halt the machine. More efficient therapeutics means better success and "leaner" treatment.

Conclusions and outlook

► In our vision we'd like to go beyond cataloging the "omics" to reach a vista of the "system" behind leukemia.

Selected Publications

1. Müller et al. (2007) Blood, 110: 4445-4454
2. Müller et al. (2009) PLOS Biology, 7(11):e1000249
3. Takacova et al. (2012) CancerCell,21(4):517-531

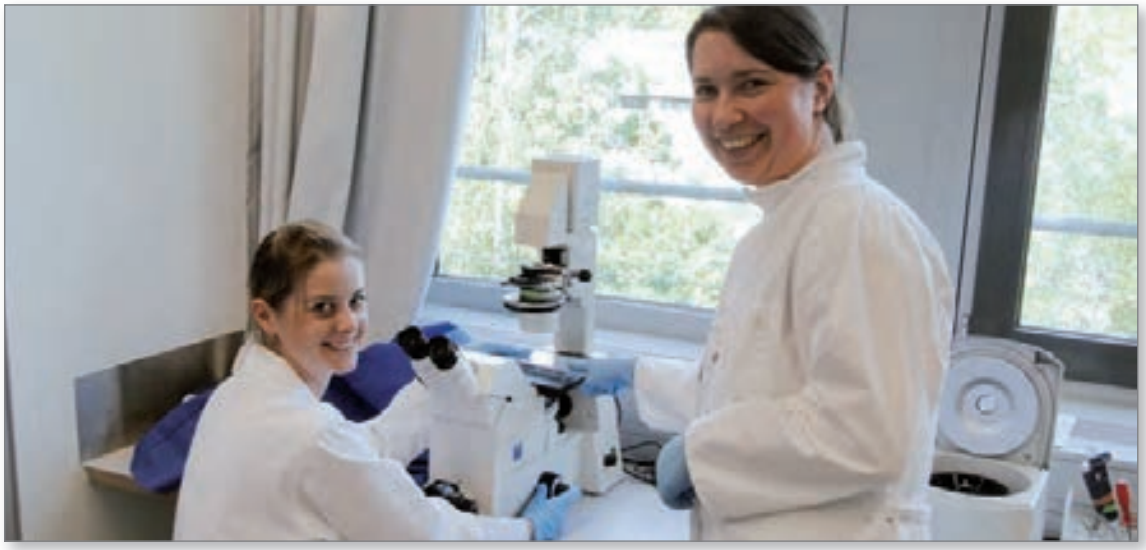

Figure 2: *In the tissue culture lab.*

Molecular Systems Analysis of Virus Genome Sensing

How is virus RNA sensed by RIG-I like innate immune receptors?

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Introduction

► The innate immune system is a first line of defense against bacterial, fungal and viral pathogens. Pattern recognition receptors sense pathogen associated molecular patterns, such as viral RNA, and elicit a host response that leads to production of e.g. interferon and proinflammatory cytokines, inducing an antiviral state of the cell. RIG-I like receptors (RLRs: RIG-I, MDA5 and LGP2) sense infections by a broad class of viruses by recognizing patterns that distinguish viral RNA from the cellular RNA. For instance, RIG-I senses 5' triphosphates on dsRNA while MDA5 is activated by long dsRNA molecules.

The innate immune system against virus infections is poorly understood on a systems level, because it is unclear how RLRs interact with viral genomes in a qualitative and quantitative manner. Although RIG-I ligands are fairly well characterized in vitro, it is unclear which RNA molecules are detected inside a living cell by different RLRs and how different RLRs cooperate to initiate a sensitive but robust antiviral response. In this project, we aim at proceeding from the structural and mechanistic knowledge of interactions of RLRs with RNA to an understanding how RLRs interact with viral and cellular RNA in virus-infected cells on a systems level. We will use next generation sequencing technologies to understand which parts of the viral genomes are detected by different RLRs, along with structure-function analysis of mutant RLRs to study how different pattern recognition sites and ATPase activities contribute to RNA pattern recognition.

Our studies should clarify which epitopes and RNA molecules are differentially detected by different RLRs in a virus specific manner and help clarify how RLRs cooperate or alternatively function in parallel or sequentially in virus sensing. For instance, mutations that trap RLRs in functional states will address whether the poorly understood ATPase activities of RLRs increase the signal-to-noise of the system. The long term goal is to understand how quantitative and qualitative interactions of RLRs are correlated with the strength of interferon production.

Selected Publications

1. Civril F, Bennett M, Moldt M, Deimling T, Witte G, Schiesser S, Carell T, Hopfner KP
The RIG-I ATPase domain structure reveals insights into ATP-dependent antiviral signalling
EMBO reports (2011) 12, 1127 - 1134

2. Myong S, Cui S, Cornish PV, Kirchhofer A, Gack MU, Jung JU, Hopfner KP, Ha T.
Cytosolic Viral Sensor RIG-I Is a 5'-Triphosphate-Dependent Translocase on Double-Stranded RNA.
Science (2009) 323, 1070-1074

3. Cui S, Eisenächer K, Kirchhofer A, Brzózka K, Lammens A, Lammens K, Fujita T, Conzelmann KK, Krug A, Hopfner KP
The C-Terminal Regulatory Domain Is the RNA 5'-Triphosphate Sensor of RIG-I. Mol Cell (2008), 169-179

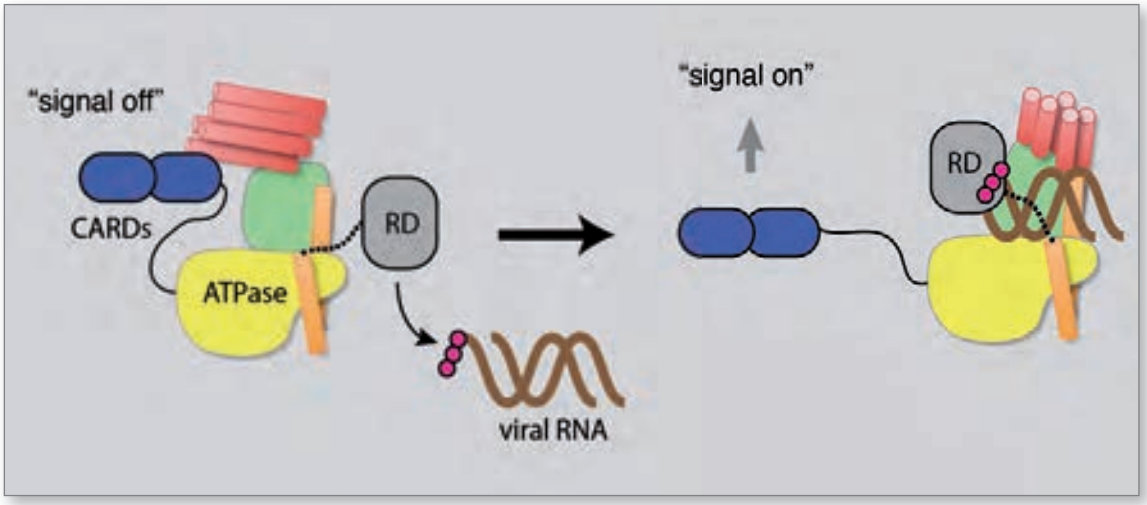

Figure 1: Structural model for the activation of the innate immune sensor RIG-I by viral RNA: 5-triphosphate RNA binds to the regulatory domain RD and induces a structural switch in the ATPase domain that liberates CARDs (caspase activation and recruitment domains) for signal transduction.

A Systems Biology Perspective on Pediatric Inflammatory Bowel Diseases

A novel approach combining clinical pediatrics, genome-wide sequence analysis, proteomic systems biology and bioinformatic tools to elucidate the pathophysiology of inflammatory bowel diseases

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Introduction

► Inflammatory bowel diseases (IBD) comprise a group of various disorders associated with chronic immune activation and tissue destruction in the intestine. Depending on clinical and pathological features, physicians classify IBD as Crohn's disease, ulcerative colitis and indeterminate colitis. The causes of IBD remain largely unknown and involve extrinsic factors (such as bacteria) and intrinsic factors (genetic susceptibility). Chronic intestinal inflammation in children results in pain, failure to grow, and multiple psychosocial problems. Existing therapeutic modalities are non-specific and often not satisfactory.

Goals of the project

► The overall objective of this project is to identify novel monogenetic disorders causing early-onset IBD, to understand basic mechanisms of immunity in the human gut, and to lay the foundation for innovative therapeutic strategies. We will delineate clinical features in pediatric patients with IBD, perform genetic studies aiming to unravel novel genetic defects causing early-onset IBD. Furthermore, we plan to generate mouse models to study the role of newly defined genes causing early-onset IBD. Finally, we plan to identify the complex interaction of defined factors and their role in the immune system.

Figure 1: *Each novel genetic defect illustrates the causes of IBD - like pieces of a complex puzzle.*

Future prospects and general impact

► To fulfill its mission, this ambitious research project is based on a genome-wide search for human disease-causing mutations and additional interdisciplinary networks to understand complex biological regulatory mechanisms. Upon identification of novel genetic factors implicated in IBD, a coordinated cooperation of biochemistry, cell biology, biophysics, and immunology is required to elucidate pathomechanisms and to provide a scientific basis for innovative therapeutic strategies for patients suffering from a devastating disease.

In the past, we identified the first truly monogenetic variants of IBD by discovering loss-of-function mutations. This discovery had implications for therapy and we successfully performed hematopoietic stem cell transplantation (HSCT), a novel therapeutic approach for selected patients with IBD. However, only 25% of all patients with early-onset IBD have mutations in these discovered genes.

Conclusions and outlook

► Studying rare patients with early-onset IBD will allow us to identify pathways controlling the homeostasis of the human intestinal immune system. A systems biology perspective bears great promise to identify novel therapeutic targets and to develop new therapeutic strategies for patients suffering from intestinal inflammation.

Selected Publications

1. Kotlarz D., Beier R., Murugan D., Diestelhorst J., Jensen O., et al. Loss of Interleukin-10 Signaling and Infantile Inflammatory Bowel Disease: Implications for Diagnosis and Therapy. Gastroenterology. 2012 (143), 347–355.

2. Glocker EO., Kotlarz D., Bozug K., Gertz EM., Schäffer AA. et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. The New England Journal of Medicine. 2009. (361), 2033-2045.

Gene-Environment Interactions

Genome-wide dissection of chromatin plasticity regulating feeding within the central nervous system of the fruit fly

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Institut Curie, Paris, France

Introduction

► For animals to survive, they must adapt to new environments. When faced with different amounts of nutrients, animals adjust their growth, cell morphology and behaviour. Different types of cells within a multicellular organism respond differently, each adapting their gene expression and physiological function to suit the new conditions. Little genome-wide information is available for these responses as determined directly within specific cell types in intact organisms. Often cell types exist in a matrix of cells that are not easily biochemically separated, such as neurons in the brain.

Goals of the project

► We will dissect how gene expression adapts within individual cell types and obtain genome-wide as well as dynamic insight into these changes. We take advantage of novel tools that we have engineered to study how changes in metabolism generate distinct gene expression responses within the brain and dissect epigenetic mechanisms that underlie these physiological adaptations.

Future prospects and general impact

► We use the powerful model organism *Drosophila melanogaster* to study how starvation and re-feeding alter chromatin dynamics and gene expression within highly specialized cell types, including neurons and fat tissue. We exploit genomic approaches, bioinformatics and modelling to measure chromatin structure, histone variants and modifications, transcription and mRNA-levels within different cell types and upon metabolic perturbation. Our long-range objectives are to identify, characterize and model epigenetic, transcriptional and behavioural mechanisms that govern an organism's systemic responses to feeding.

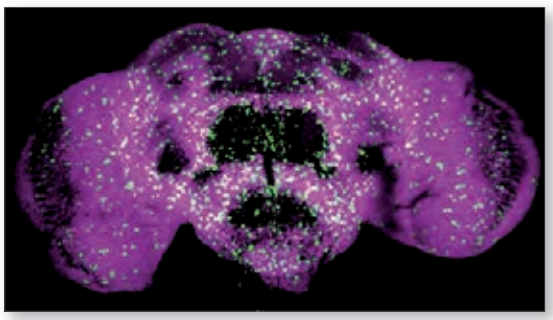

Figure 1: *The Drosophila brain imaged with markers for glia (green) and neurons (magenta).*

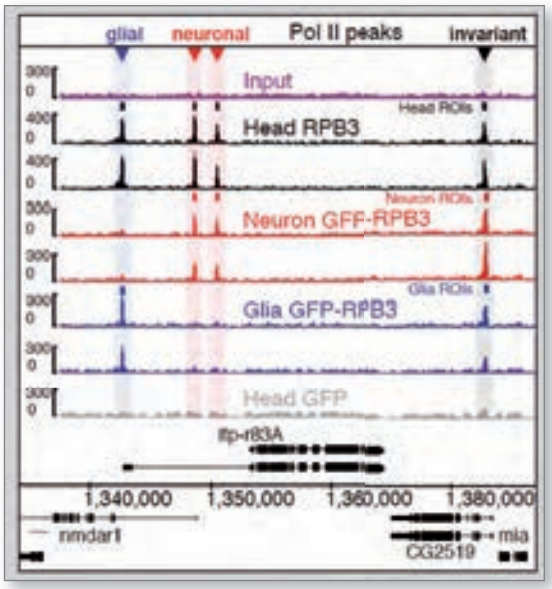

Figure 2: *Mapping active genes at genome-wide resolution within specific cell types of the fly brain.*

Conclusions and outlook

► Our analysis will provide novel spatial and temporal resolution of gene-environment interactions at the level of gene regulation, promising to reveal fundamental new insight into mechanisms regulating metabolism and feeding behaviour. Considering the range of nutritional disorders that exist in humans (e.g. obesity, addiction, compulsive behaviours), the project is relevant to human health, since many signalling pathways have first been described in model organisms (e.g. circadian clock, long-term memory) including the fly. The use of state-of-the-art methods, novel tools and a powerful genetic and behavioural organism will allow us to identify and validate new mechanisms relevant to the connections between metabolism, genes and behaviour.

Selected Publications

1. Jankevicius, Hassler, Golia, Rybin, Zacharias, Timinszky and Ladurner (2013). A family of macrodomain proteins reverses cellular mono-ADP-ribosylation. Nature Structural & Molecular Biology 20(4), 508-514.

2. Ali, Timinszky, Arribas-Bosacoma, Kozlowski, Hassa, Hassler, Ladurner, Pearl and Oliver (2012). The zinc-finger domains of PARP1 cooperate to recognize DNA strand breaks. Nature Structural & Molecular Biology 19, 685-692.

Developmental Impact of Maternal Diabetes Mellitus

A molecular systems study of oocytes, embryos and their maternal environment in genetically designed diabetic mouse and pig models

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Introduction

► The global prevalence of diabetes mellitus is dramatically increasing. Maternal diabetes is known to adversely affect embryo development and pregnancy outcomes. These effects may be associated with structural, functional or molecular changes, at different levels, from compromised oocyte competence to alterations of the uterine environment.

Goals of the project

► To integrate quantitative molecular, structural and functional parameters indicating effects of maternal hyperglycemia on oocyte developmental competence

► To provide insights into developmental pathways affected by maternal hyperglycemia and their regulation by epigenetic mechanisms

► To identify molecular changes within the maternal-fetal crosstalk in the diabetic condition

We will use corresponding mouse and pig models expressing mutant insulin molecules, leading to permanent hyperglycemia. Oocytes and embryos recovered from these models will be analyzed for structural, functional and molecular abnormalities as compared to control oocytes. In addition, we will perform expression profiling of the uterine endometrium to evaluate effects of hyperglycemia on maternal recognition of pregnancy.

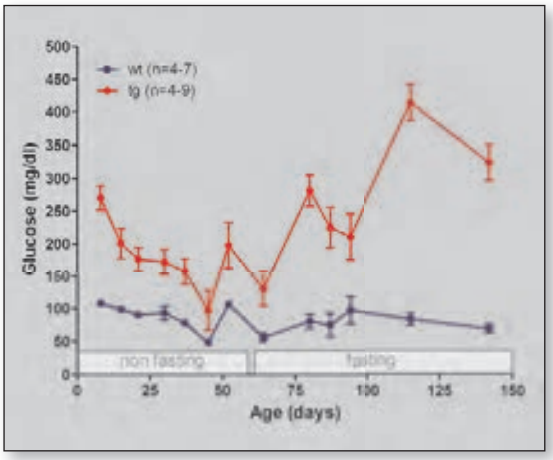

Figure 1: *Permanent diabetes in INSC94Y transgenic pigs (tg) as compared to wild-type controls (wt).*

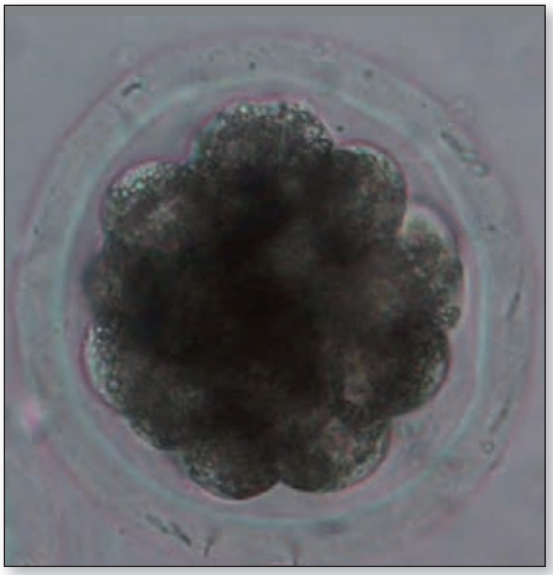

Figure 2: *Porcine embryo at the morula stage.*

Conclusions and outlook

► These studies will for the first time provide systematic insights into developmental consequences of maternal hyperglycemia, including the pig as a relevant model for early human development. Identification of critical windows in development with high susceptibility for maternal diabetes may help to refine preventive strategies to avoid developmental failures and consequences for future health.

Selected Publications

1. Renner S, Fehlings C, Herbach N, Hofmann A, von Waldthausen DC, Kessler B, Ulrichs K, Chodnevskaja I, Moskalenko V, Amselgruber W, Göke B, Pfeifer A, Wanke R, Wolf E. Glucose intolerance and reduced proliferation of pancreatic beta-cells in transgenic pigs with impaired glucose-dependent insulinotropic polypeptide function. Diabetes. 2010 May;59(5):1228-38.

2. Klymiuk N, van Buerck L, Bähr A, Offers M, Kessler B, Wuensch A, Kurome M, Thormann M, Lochner K, Nagashima H, Herbach N, Wanke R, Seissler J, Wolf E. Xenografted islet cell clusters from INSLEA29Y transgenic pigs rescue diabetes and prevent immune rejection in humanized mice. Diabetes 2012 Jun;61(6):1527-32.

3. Renner S, Römisch-Margl W, Prehn C, Krebs S, Adamski J, Göke B, Blum H, Suhre K, Roscher AA, Wolf E. Changing metabolic signatures of amino acids and lipids during the prediabetic period in a pig model with impaired incretin function and reduced β-cell mass. Diabetes 2012 Aug;61(8):2166-75.

Exploring and Explaining Expression Patterns

Regulatory networks involved in host response to Herpes virus infection

Introduction

► The Exp3 project develops an iterative approach for exploring and explaining expression patterns in regulatory networks using targeted combinatorial perturbations (multiple siRNA knockouts/knockdowns) of genes and sets of genes followed by the subsequent gene and protein expression measurements of the perturbed system.

Goals of the project

► From network, interaction and process models a number of suitable and informative perturbations are proposed, which will be applied via combinatorial knockdown of one or multiple genes, followed by functional assays for virus replication and a detailed measurement of the expression levels of a large number of relevant genes (again proposed via the models). Causal, time and space resolved executable (Petri Nets with Fuzzy Logic = PNFL) models are derived and exploited for the prediction of new target sets. This Exp3 systems approach is applied for the investigation of Herpes virus infections to identify relevant virus host factors and allows for investigating specific innate and adaptive immune response pathways challenged by Herpes viruses in a much more in-depth way as previously possible. Moreover, we consider the combinatorial regulatory effects of (Herpes viral) miRNAs and siRNA and their effect on protein isoforms and their structures.
Figure 1 shows various high throughput data, which have already been measured and which will be used to construct initial models and to predict the first round of perturbations. Figure 2 visualizes a current view on a target pathway associated with the immune response of the host cell. Using public and own data on PPIs and regulatory interactions a much more comprehensive and dense network is obtained.

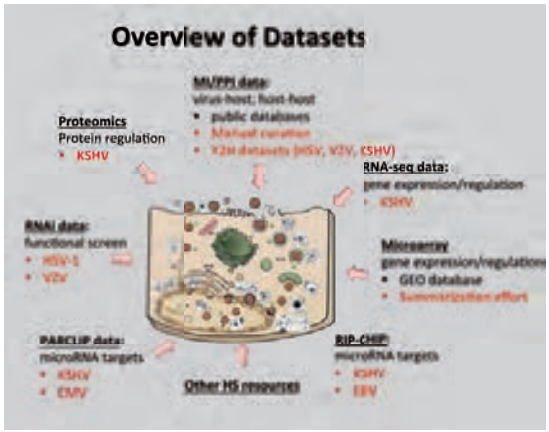

Figure 1: Overview of experimental data for the Herpes system.

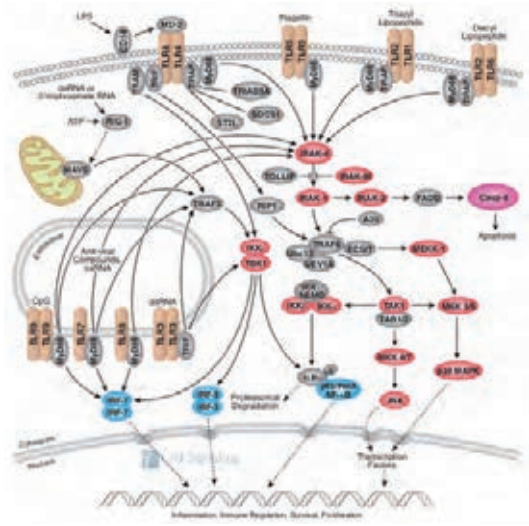

Figure 2: Visualization of a textbook innate immune response pathway relevant for viral infections [Source: http://www.cellsignal.com/reference/pathway/Toll_Like.html].

Thus, many more edges between the players of the target pathways are available, in several cases also with additional annotations (direction, sign, strength, confidence) of interactions. The Exp3 method aims at transforming such coarse networks employing targeted high throughput measurements into executable (PNFL) models explaining the data and describing the biological system in a qualitative and (semi-)quantitative way.

Future prospects and general impact

► The Exp3 method should save time and money in discovering new regulatory mechanisms.

Conclusions and outlook

► The proposed approach is applicable to a wide range of systems modeling problems by drastically reducing the number of experiments yielding much more focused experimental data for obtaining causal models. As the approach is predictive it will also allow for proposing validation experiments, which can be performed via the very same protocol.

Selected Publications

1. Marcinowski L, Lidschreiber M, Windhager L, Rieder M, Bosse JB, Rädle B, Bonfert T, György I, de Graaf M, Prazeres da Costa O, Rosenstiel P, Friedel CC, Zimmer R, Ruzsics Z, Dölken L. PLoS Pathog. 2012 Sep;8(9):e1002908.
2. Marbach D, Costello JC, Küffner R, Vega NM, Prill RJ, Camacho DM, Allison KR; DREAM5 Consortium, Kellis M, Collins JJ, Stolovitzky G. Nat Methods. 2012 Jul 15;9(8):796-804. doi: 10.1038/nmeth.2016.
3. Windhager L, Bonfert T, Burger K, Ruzsics Z, Krebs S, Kaufmann S, Malterer G, L'hernault A, Schilhabel M, Schreiber S, Rosenstiel P, Zimmer R, Eick D, Friedel CC, Dölken L. Genome Res. 2012 Oct;22(10):2031-42. doi: 10.1101/gr.131847.111.

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Cooperative miRNA Function in Cardiovascular Disease

Understanding the mechanisms of microRNA interaction may contribute to the development of novel therapeutic strategies in cardiovascular disease

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Introduction

► MicroRNAs (miRNAs) are small non-coding RNAs with a length of 20 to 30 nucleotides that post-transcriptionally regulate target genes by base-pairing with complementary sequences in the 3′ untranslated regions (UTRs) of protein-coding transcripts. MiRNAs are required for virtually all phases in the life of a living cell or organism. Although a growing number of miRNAs has been assigned a critical role in the pathogenesis of several diseases including those of the cardiovascular system, the mechanism of action of many miRNAs remains unclear. One important obstacle towards a better understanding of miRNA function stems from the intrinsic mechanism of action of miRNAs: next to their embedding into their functional unit, the miRISC ribonucleoprotein complex, only 6-8 nucleotides of an miRNA determine mRNA recognition.

Goals of the project

Given this, bioinformatic analyses predict a plethora of target mRNAs, interactions and levels of regulation: i) almost every mRNA contains miRNA binding sites, ii) various mRNAs may carry sites for an individual miRNA, and iii) one mRNA may contain sites for various miRNAs. Based on these predictions we may expect an enormous complexity in miRNA-dependent regulatory networks. This complexity could be required to fine-tune or to boost the cellular response to an external stimulus - either on a single mRNA or through cascades of miRNA regulation on different levels within a pathway. The topic of this BioSysNet research group is the functional characterization of cardiovascular miRNAs and their interactions in cardiovascular disease. Elucidating the underlying mechanisms may provide an entry point for therapeutic intervention.

Selected Publications

1. Jentzsch C, Leierseder S, Loyer X, Flohrschütz I, Sassi Y, Hartmann D, Thum T, Laggerbauer B, Engelhardt S. A phenotypic screen to identify hypertrophy-modulating microRNAs in primary cardiomyocytes. J Mol Cell Cardiol. 2012;52(1):13-20.
2. Thum T, Chau N, Bhat B, Gupta SK, Linsley PS, Bauersachs J, Engelhardt S. Comparison of different miR-21 inhibitor chemistries in a cardiac disease model (Letter). J Clin Invest. 2011;121(2):461-2
3. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature. 2008;456(7224):980-4.

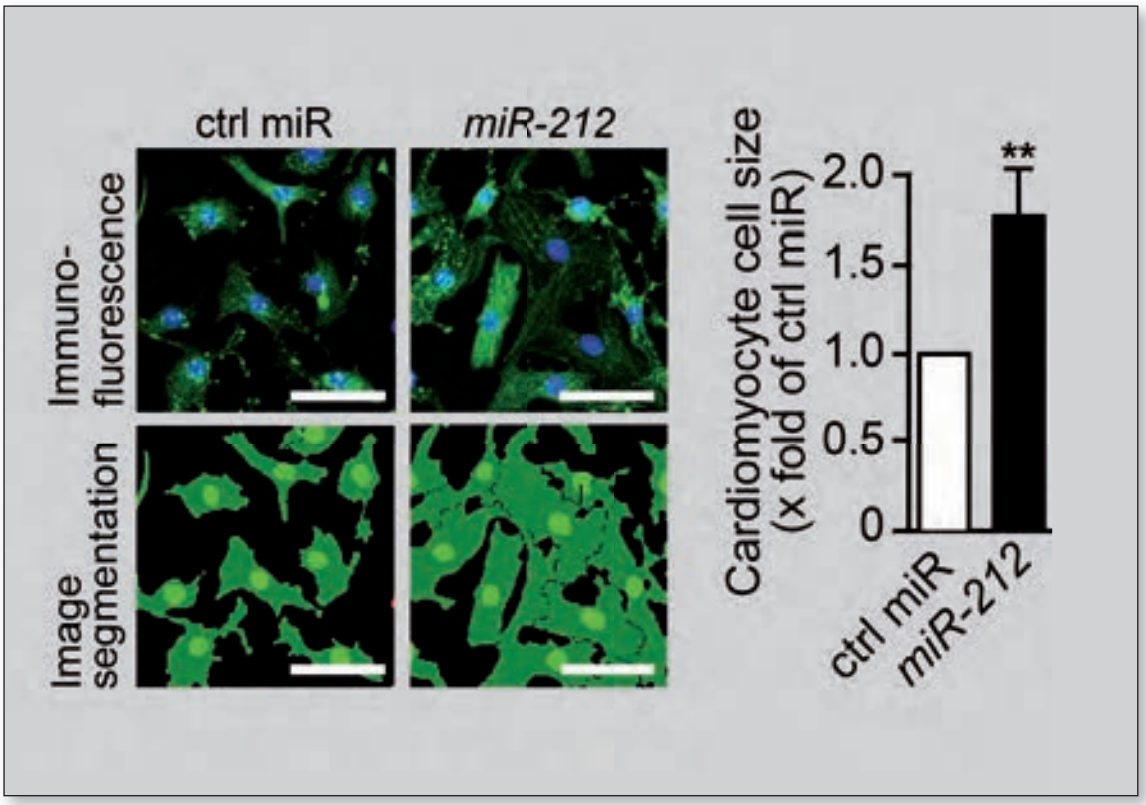

Figure 1: *MicroRNA-212 regulates cardiomyocyte cell size.*

Molecular Understanding of Malignant Melanoma - Lessons from Embryogenesis

Understanding early diferentiation of melanoblasts will help to define molecular events in melanoma development

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Introduction

► Cells in embryogenesis harbor several features such as high proliferative capability, which are also found in cancer cells. In embryogenesis these characteristics are strictly controlled whereas in cancer this regulation is lost. In this project we will focus on a detailed understanding of differentiation and migration of melanocytes in embryogenesis compared to melanoma cells to determine molecular changes in regulation.

Goals of the project

► Melanoma is an aggressively disseminating cancer with high incidence still continuously rising. Melanoma cells derive from melanocytes, which originate from the neural crest and display several characteristics of neuronal cells. During embryogenesis melanoblasts, the precursors of melanocytes, reveal several "tumorigenic" features as they migrate actively and subsequently "invade" the epidermis. These findings lead to our hypothesis, that only few but critical molecular alterations allow melanoma cells to "reinitiate and exploit" characteristics of melanoblasts, and therefore, a detailed understanding of neural crest differentiation and melanoblast migration will lead to crucial novel information about melanoma development and progression. The main aims derived from the hypothesis are: 1. Detection of molecules which can induce differentiation of melanoma cells and 2. Determination of molecular differences between melanoblasts, melanocytes and melanoma cells.

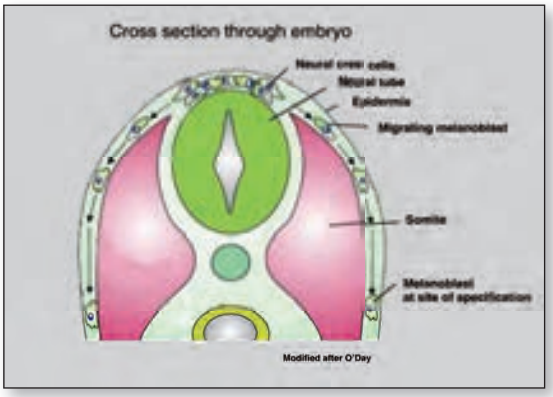

Figure 1: Melanoblast migration in embryogenesis.

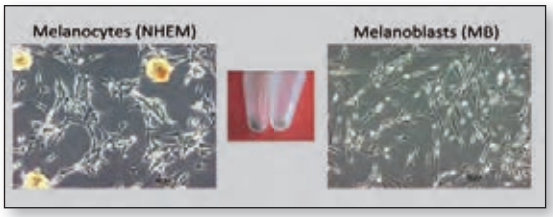

Figure 2: Phenotypic analysis of melanoblasts in cell culture. Macroscopic and microscopic differences in pigmentation are clearly visible.

Conclusions and outlook

► In general, our experimental approaches are designed to discover novel central factors for melanoma development and progression, which have not been associated with the pathogenesis of melanoma before, and will result in a better molecular and cellular understanding of this biological system. In the long term, new targets for effective melanoma therapy could be determined.

Selected Publications

1. Bosserhoff AK, Ellmann L, Kuphal S: Melanoblasts in culture as an in vitro system to analyze melanoma. Exp Dermatol 20: 435-440 (2011)
2. Massoumi R, Kuphal S, Hellerbrand C, Haas B, Wild P, Spruss T, Pfeifer A, Fassler R, Bosserhoff AK: Down-regulation of CYLD expression by Snail promotes tumor progression in malignant melanoma. J Exp Med 206: 221-232 (2009)
3. Schmidt J, Friebel K, Schönherr R, Coppolino MG, Bosserhoff AK: Directed, migration-associated secretion of MIA at the cell rear regulates melanoma cell mobility. Cell Res 20: 1224-1238 (2010)

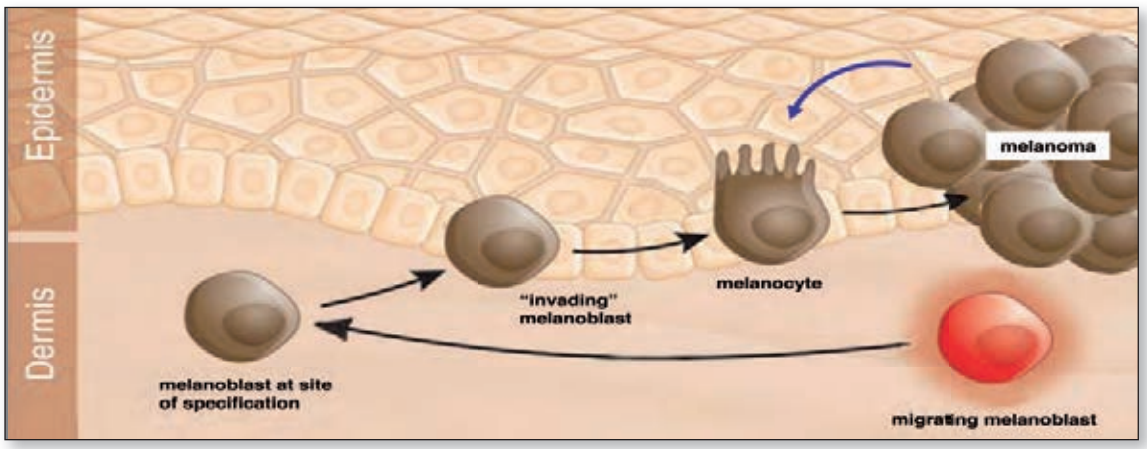

Figure 3: Schematic illustration of the differentiation and de-differentiation processes.

Proteomics-Based Identification of Cellular Networks Regulating miRNA Maturation

Regulation of miRNA processing by RNA-binding proteins

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Introduction

► MicroRNAs (miRNAs) are a class of small noncoding RNAs that negatively regulate gene expression by destabilization or translational inhibition of mRNAs. MiRNAs are encoded in the genome of almost all eukaryotes and are synthesized as part of longer primary transcripts. Such transcripts are subsequently processed by two nucleolytic events catalyzed by the RNase III enzymes Drosha and Dicer. The expression of miRNAs is tightly regulated, and in many cases deregulation of miRNA abundance has been linked to diseases including cancer. Such regulatory events are for example transcriptional regulation, miRNA degradation or interaction of RNA-binding proteins with the different miRNA processing intermediates thus preventing them from processing.

Goals of the project

► Based on several studies, it has become evident that the maturation steps of miRNAs can be positively or negatively regulated by RNA binding proteins. However, only a small number of posttranscriptional miRNA regulators has been discovered to date. Therefore, the aim of our project is the identification of RNA-binding proteins regulating miRNA processing. We have set up a proteomics-based screen to identify proteins specifically binding to individual miRNA precursors. In the proposed project, we plan to screen a set of about 100 different miRNAs in 10 different cancer cell lines in order to identify posttranscriptional miRNA regulators involved in tumorigenesis and tumor progression.

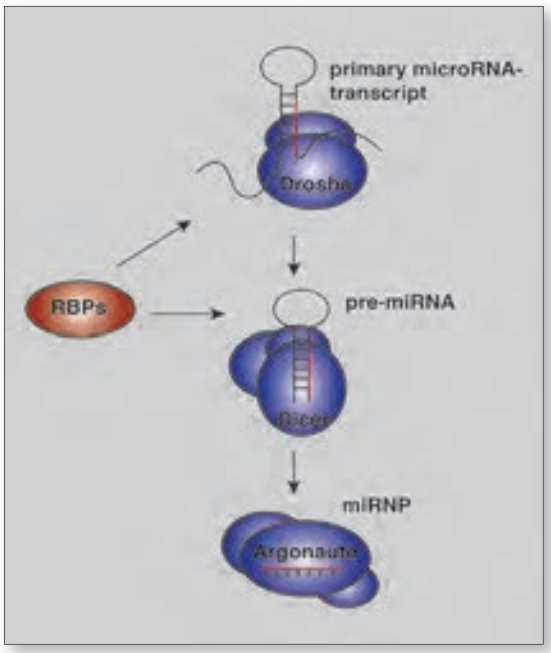

Figure 1: *Regulation of miRNA maturation by RNA-binding proteins. MiRNAs are synthesized as primary transcripts that are processed to miRNA precursors by Drosha. These molecules are further processed to mature miRNAs by Dicer. Mature miRNAs bind to a member of the Argonaute protein family and are incorporated into miRNA-protein complexes termed miRNPs. In this project, we aim at the identification of RNA-binding proteins (RBPs) that bind to miRNA precursors and regulate miRNA expression.*

Future prospects and economic impact

► miRNAs are important for various cellular functions and consequently, miRNAs are frequently misregulated in many diseases. Our work will contribute to a better understanding of the causes that lead to miRNA misregulation and therefore also to a better understanding of the establishment of diseases like cancer. Modulating such regulatory networks might also have an economic impact in the future.

Conclusions and outlook

► Proteins containing putative RNA-binding domains are very abundant in the human genome, many of them with unknown functions. MiRNA expression is regulated at many different steps including binding of processing intermediates by RNA-binding proteins. We hypothesize that many miRNA families might be under the control of specific RNA-binding proteins and many of the so far uncharacterized RNA-binding proteins might be involved in miRNA regulation.

Selected Publications

1. Schraivogel D., Weinmann L., Beier D., Tabatabai G., Eichner A., Zhu J.Y., Anton M., Sixt M., Weller M., Beier C. & Meister G. (2011) CAMTA1 is a novel tumor suppressor regulated by miR-9/9* in glioblastoma stem cells, EMBO J., 30(20):4309-22.

2. Weinmann, L., Höck, J., Ivacevic T., Ohrt T., Mütze J., Schwille P., Kremmer E., Benes V., Urlaub, H., & Meister G. (2009) Importin 8 is a gene silencing factor that targets Argonaute proteins to distinct mRNAs. Cell, 136, 496-507.

3. Ender C., Krek A., Friedländer M.R., Beitzinger M., Weinmann L., Chen W., Pfeffer S., Rajewsky N. & Meister G. (2008) A human snoRNA with microRNA-like functions. Molecular Cell, 32, 519-528.

Modelling Paracrine Communication

Towards a map of intercellular gossip

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Introduction

► Cells of different origin in a tissue communicate via secreted proteins. Immune cells communicate with virtually all cells of the body. Stromal cells communicate with tumor cells and the signals they give modulate tumor growth, migration and the resistance to treatment. While this cross talk certainly involves feedback mechanisms in vivo, we developed experimental setups in which unidirectional signaling is guaranteed. Hence in our experimental setup there is always a sender cell and a receiver cell. The sender emits signaling molecules and the receiver has receptors that sense these molecules and its inner signaling network orchestrates the response to the signals from the environment.

Goals of the project

► From a systems biology perspective, intercellular communication links the molecular networks of different cell types. We design computational models that capture the cross talk of cellular networks. Our focus is on causal relationships between the composition of the secreted signaling molecules and the receiver cell's response. For all secreted molecules we computationally predict how increasing its amount in the secretome by one unit will change the receiver's response. In collaboration with the labs of Prof. Claus Hellerbrand and Prof. Peter Oefner, we grow various lines of sending cells separate from the receiving cells and use the conditioned media of these cells to stimulate receiver cells. We characterize the transcriptomes of sending cells, the content of the conditioned media and the transcriptomes of stimulated receiver cells. The observed statistical dependencies between these measurements drive our model. Our primary application will be to understand the cross talk of hepatocellular carcinoma cells and stromal cells in the cancer tissue.

Future prospects and economic impact

► Our long term vision is that once we understand how the composition of the extracellular proteome in a tumour shapes the molecular networks and transcriptome of a tumour cell, new avenues to design targeted combination therapies will open. More concretely, we envision therapies combining existing anti-cancer drugs with certain growth factors or inhibitors thereof that help sensitize the tumour to the drug. While this is a long term goal, we believe that first steps in this direction include the development of experimental frameworks and computational methodology to investigate paracrine signalling systematically on the systems level.

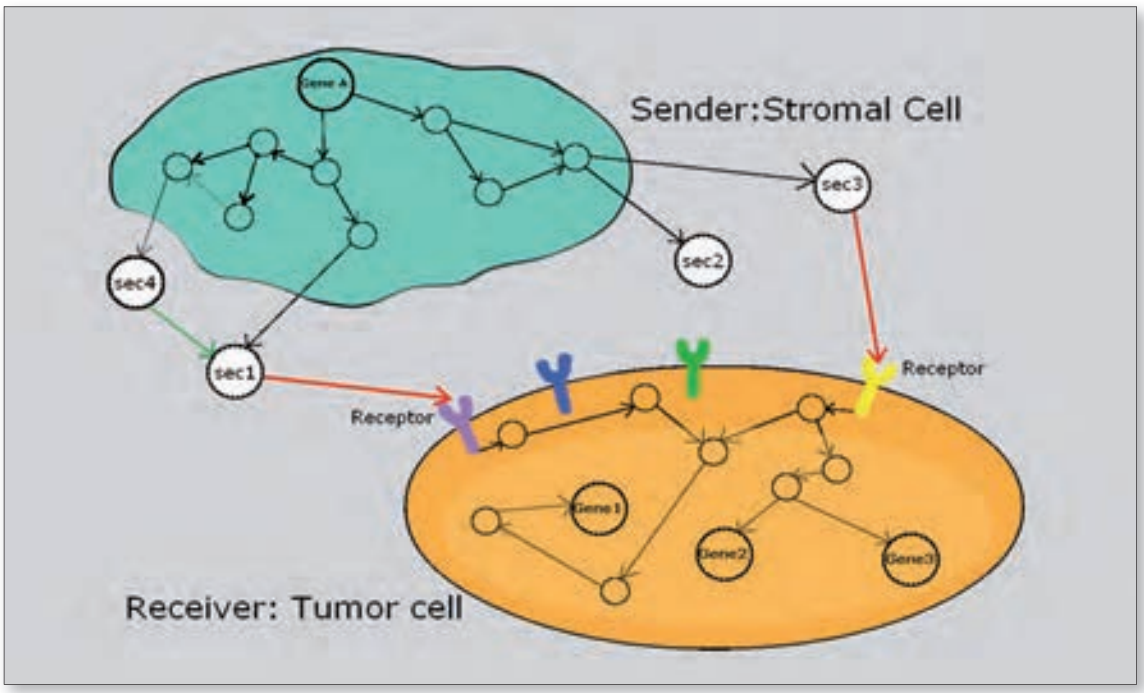

Figure 1: *Paracrine Communication: The molecular network of a sending cell determines the composition of a complex secretome that simultaneously passes multiple messages to a receiving cell. The receiver cell's intracellular networks in turn process these inputs and adapt the receiver's phenotype.*

Temporal Control of Gene Expression by Small RNAs

A systems biology approach to understanding the principles of small noncoding RNAs in bacteria

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Introduction

► Small noncoding RNAs (sRNAs) have begun to rival transcriptional factors with regards to numbers and regulatory scope in gene expression. Most bacterial sRNAs regulate multiple mRNAs by short seed pairing, and it is now well-established that many govern large post-transcriptional regulons. We know little about how sRNAs differ from transcription factors in terms of regulatory activities and biological meaning, but increasing evidence suggests that they impact timing and threshold behaviour in gene expression programs.

Goals of the project

► This project aims to understand how sRNAs shape the dynamics of global responses in the pathogenic model organism, *Salmonella Typhimurium*. We will focus primarily on the SigmaE-induced envelope stress response, but also investigate the SOS response to DNA damage, the stringent response to starvation, and the regulatory cascades that induces *Salmonella's* genes for host cell invasion.

Conclusions and outlook

► We will harness the power of RNA deep sequencing and in vivo cross-linking for genome-wide profiling of regulation at the levels of DNA and RNA in parallel. This will unravel temporal and directional differences in the regulation of individual genes at the transcriptional versus post-transcriptional level, and identify network motifs that interconnect these two layers in natural systems. Knowledge of these motifs will feed into a rational design of sRNAs that function as timing devices in synthetic control circuits.

Selected Publications

1. Chao Y, Papenfort K, Reinhardt R, Sharma CM, Vogel J (2012) An atlas of Hfq-bound transcripts reveals 3′ UTRs as a genomic reservoir of regulatory small RNAs EMBO Journal 31(20)4005-19

2. Papenfort K, Podkaminski D, Hinton JC, Vogel J (2012) The ancestral SgrS RNA discriminates horizontally acquired Salmonella mRNAs through a single G-U wobble pair PNAS 109(13):E757-64

3. Sharma CM, Hoffmann S, Darfeuille F, Reignier J, Findeiß S, Sittka A, Chabas S, Reiche K, Hackermüller J, Reinhardt R, Stadler PF, Vogel J (2010) The primary transcriptome of the major human pathogen Helicobacter pylori Nature 464(7286):250-255

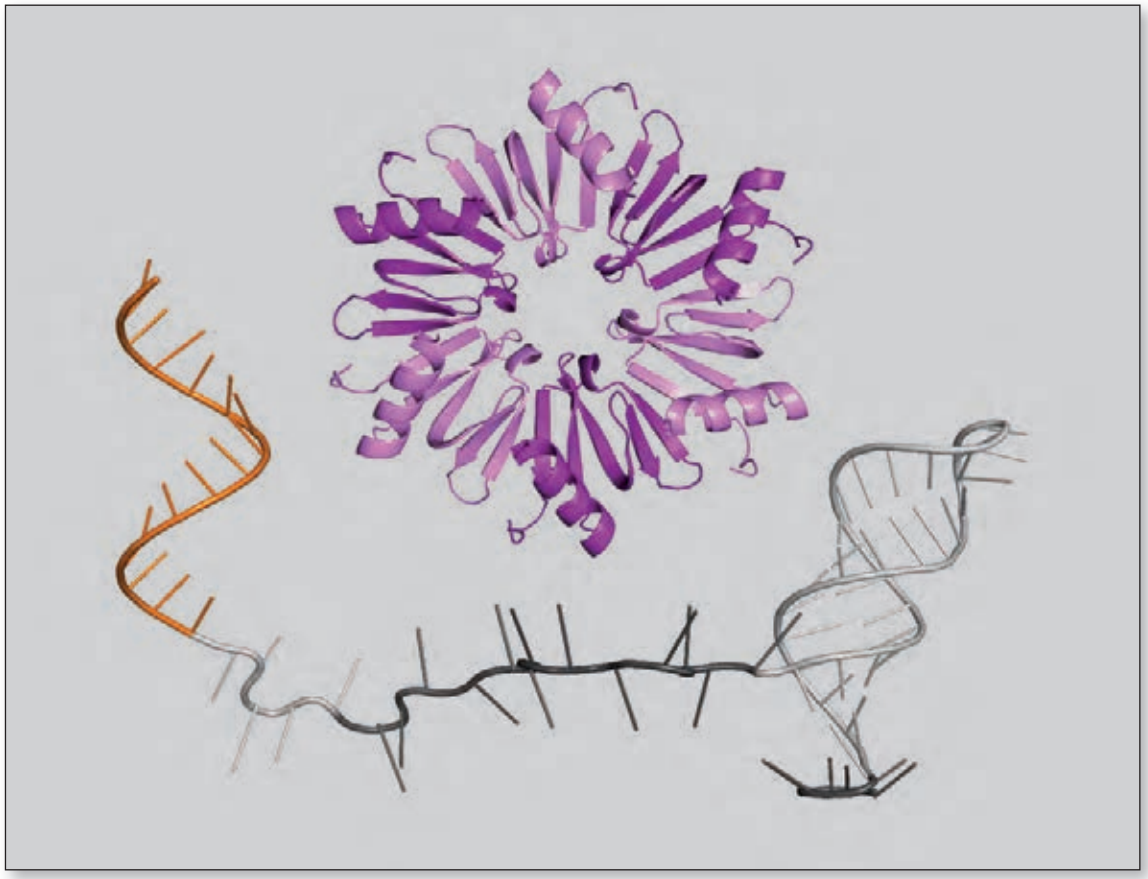

Figure 1: *A bacterial small noncoding RNA and the RNA chaperone Hfq.*

Care-for-Rare Foundation

Patients with rare diseases present a particular challenge for medicine – diagnosis is difficult and for the majority of affected patients no cure is available. Mutations in the genome often and inevitably lead to early death – sometimes even in the first few years of life. Thus, research is of vital importance to shed light on the pathomechanisms and to lay the foundations for the development of urgently needed therapies.

The Care-for-Rare Foundation for children with rare diseases is taking on those challenges. Supported by Nobel Laureates and under the patronage of Federal Minister for Education and Research, Annette Schavan, the Foundation follows the mission that no child should die of a devastating rare disease – independent of ethnicity, religion, or wealth.

Thanks to the Care-for-Rare Foundation, many children throughout the world have been diagnosed with newly discovered genetic diseases and many could be rescued by use of novel therapeutic strategies. Visit the Foundation's website at **www.care-for-rare.org** for more details. Donations will go directly to children in need and research projects – without any deduction. Our biannual newsletter will inform you about our ongoing and completed projects.

Your donation is highly appreciated!

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Care-for-Rare Foundation
Foundation for Children with Rare Diseases

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Help us help!

HSP is Hereditary Spastic Paraplegia, a rare and incurable hereditary motor neuron disease with childhood or young adult onset that constantly gets worse. HSP – those three letters make walking difficult and can imply life in a wheelchair. Between 2000 and 3000 people suffer from Hereditary Spastic Paraplegia in Germany.

This is why in 1998, Dr. Tom Wahlig from Münster in Westfalia, whose son Henry has also suffered from HSP since childhood, established the foundation named after him. Since 1998, the Tom Wahlig Foundation has set up HSP-consultation in cooperation with many hospitals throughout Germany and Austria to help facilitate the beginning of this path. The core assignments of the Tom Wahlig Foundation include promoting research on HSP through start-up funding of projects, awarding research scholarships and building networks by means of our symposium.

When the Tom Wahlig Foundation was established in 1998, none of the genes which, when mutated can cause HSP, were known. By now, around 50 gene loci and close to 20 genes have been identified. The hope of causal therapy linked to these discoveries has yet to be fulfilled. One of the BioSysNet project leaders, Dr. Beate Winner, and her colleagues are currently working with the support of Tom Wahlig Foundation research scholarships to create human HSP neurons from skin cells.

HSP is not curable – yet. The work of medical doctors, neuroscientists, and researches from other areas of expertise gives those affected by HSP daily hope that a way to cure or to alleviate the effects of this disease will be found. Since 1998, the foundation has been able to significantly advance basic research in HSP - after having remained static for close to 100 years. Also many new insights were gained by means of the Tom Wahlig Foundation support.

Help us help!
Help those affected by HSP by contributing to a vision of a future without HSP. The foundation will support you in this quest by all available means!

http://www.hsp-info.de

Cooperation Partners in the Industry

The Bavarian Reserach Network for Molecular Biosystems supports collaboration with academic research groups outside the network from Germany and around the world. Cooperation with industrial partners are also aspired as both sides will benefit from it.

Additonal partners are:

IDT - Integrated DNA Technologies,

2bind

BioSysNet

Bavarian Research Network for Molecular Biosystems

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Aufbruch Bayern

Bayerisches Staatsministerium für Wissenschaft, Forschung und Kunst